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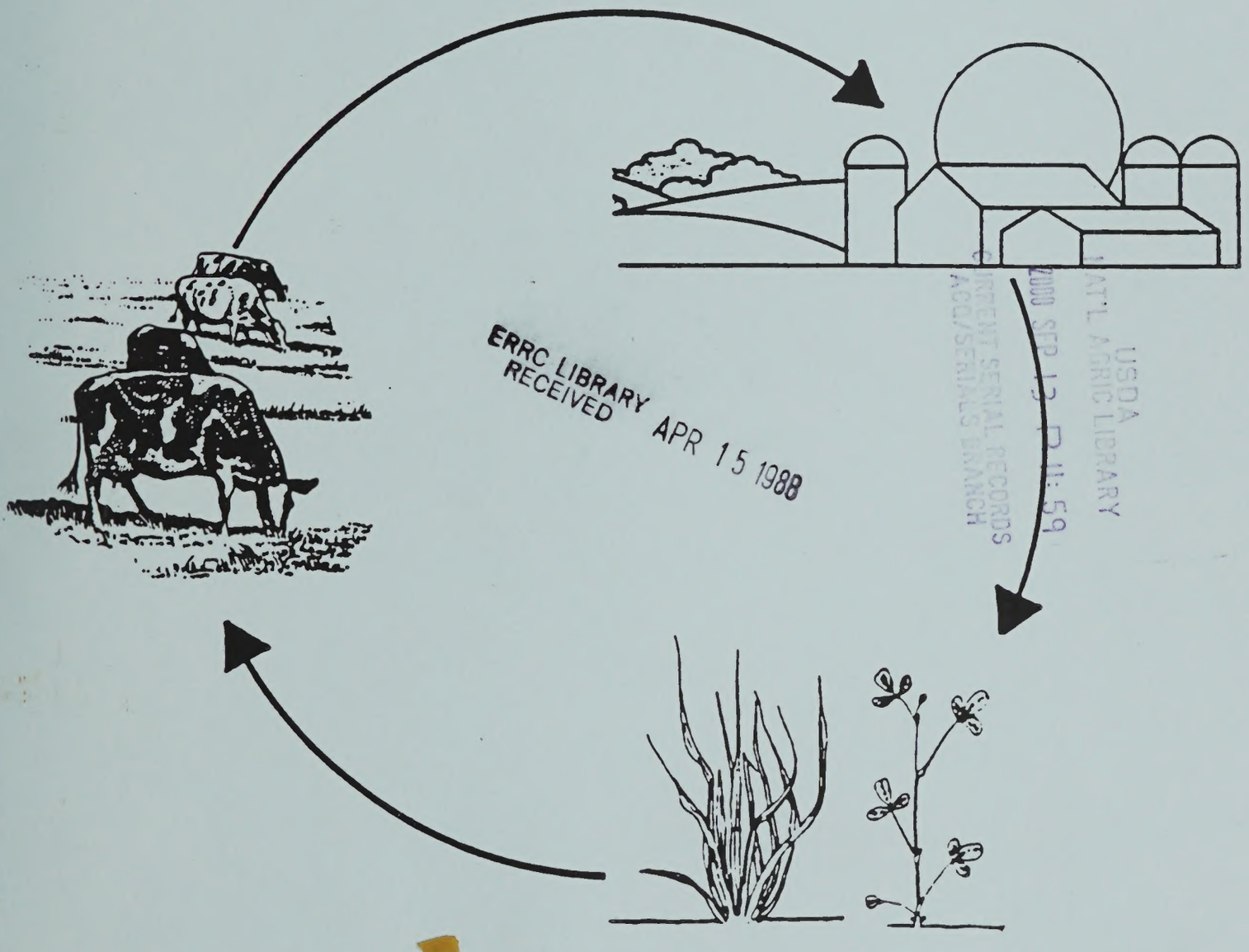
US DAIRY FORAGE RESEARCH CENTER

1987 RESEARCH SUMMARIES



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Agricultural Research Service

United States Department of Agriculture

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March 1988

U.S. DAIRY FORAGE RESEARCH CENTER, ARS-USDA
Madison, WI 53706

Dear Reader:

It is a pleasure to update our progress by bringing you these summaries of recent research. The U.S. Dairy Forage Research Center is a unique part of the national research program of the Agricultural Research Service, U.S. Department of Agriculture. The Center's mission is to build a knowledge and technology base for the dairy industry to fully exploit the use of forages in the production of milk. The Center has agricultural engineers, plant and soil scientists, microbiologists, and ruminant nutritionists working together to increase the efficiency of forage production and utilization by dairy farmers. We function in close cooperation with the Agricutural Experiment Stations of several states. The Center is located on the campus of the University of Wisconsin, Madison, and has "Cluster" locations in St. Paul, MN, Ames, IA, Columbia, MO, Wooster, OH, East Lansing, MI, University Park, PA, and Ithaca, NY. The Center's research farm, with facilities for 300 milking cows, is located on 63 acres of USDA land on the banks of the Wisconsin River in Prarie du Sac, WI. An additional 1200 acres of adjacent land is utilized by the Center by agreement with the U.S. Department of the Army.

The Center was established in 1980 and has made steady growth since. At present there are sixteen scientists; eight at Madison, and one each at six of the Cluster locations, and two at the St. Paul, Minnesota Cluster location. We expect Dr. Paul Weimer, currently with DuPont, to arrive in Madison sometime in April or May to fill our microbiologist position. Dr. John Ralph, currently in the Chemistry Department at the University of California-Berkeley, will be coming about the same time to fill the Chemist position. We are very pleased to have these two outstanding individuals join our staff. We have resources to fund a temporary systems analyst position, but we are in the process of re-evaluating this position. Reductions in federal spending are causing us to carefully scrutinize every staffing change.

The selection process for a new director to replace Dr. Charles Kiddy who retired at the end of 1986 culminated in October. I was named Director effective November 8. I am excited about the challenges and the opportunities that we have as a Dairy-Forage Research Center and I welcome your suggestions and support as we work together to serve the dairy-forage interests of our nation.

We are pleased and very proud of the way Center scientists from diverse disciplines interact and bring their collective insights to bear on the problems of forage production and utilization. This collection of research summaries illustrates the progress they are making in developing information to help dairy farmers utilize their resources more effectively. The research is intended to benefit dairy farmers and the consumers of dairy products.

Sincerely,

Larry D. Satter

Larry D. Satter, Director
U.S. Dairy Forage Research Center

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U.S. DAIRY FORAGE RESEARCH CENTER STAFF

S.M. Abrams
Research Animal Scientist
USDA/ARS/USDFRC
USDA Regional Pasture Research Lab
Pennsylvania State Universtiy
University Park, PA 16802
(814) 836-0984

*G.A. Broderick
Research Dairy Scientist
(608) 263-6824
FTS: 364-5356

D.R. Buxton
Research Plant Physiologist
USDA/ARS/USDFRC
Department of Agronomy
Iowa State University
Ames, IA 50011
(515) 294-9654
FTS: 865-9654

*R.D. Hatfield
Research Plant Physiologist
(608) 263-9627
FTS: 364-5358

H.G. Jung
Research Dairy Scientist
USDA/ARS/USDFRC
Animal Science Department
Room 130, Haecker Hall
University of Minnesota
St. Paul, MN 55108
(612) 624-7753

*R.G. Koegel
Research Agricultural Engineer
(608) 263-5636
FTS: 364-5149

F.A. Martz
Research Dairy Scientist
USDA/ARS/USDFRC
Department of Dairy Science
University of Missouri
Columbia, MO 65211
(314) 882-1138
FTS: 276-5356

*D.R. Mertens
Research Dairy Scientist
(608) 263-1558
FTS: 364-5228

*R.E. Muck
Research Agricultural Engineer
(608) 263-3742
FTS: 364-5245

C.A. Rotz
Research Agricultural Engineer
USDA/ARS/USDFRC
Rm 206, Agric. Engr. Dept.
Michigan State University
East Lansing, MI 48824
(517) 353-1758
FTS: 374-6706

J.B. Russell
Research Microbiologist
321 Morrison Hall
Department of Animal Science
Cornell University
Ithaca, NY 14853
(607) 255-4508

M.P. Russelle
Soil Scientist
USDA/ARS/USDFRC
Department of Soil Science
University of Minnesota
1529 Gortner Avenue
St. Paul, MN 55108
(612) 787-3242

*L.D. Satter
Director &
Research Dairy Scientist
(608) 263-2030

W.L. Shockey
Research Dairy Scientist
USDA/ARS/USDFRC
Department of Dairy Science
103 Gerlaugh Hall
Ohio Agricultural Research
& Development Center
Wooster, OH 44691
(216) 263-3794

*R.R. Smith
Research Plant Geneticist
(608) 262-9752
FTS: 364-5279

L.L. Strozinski
Herd Manager
USDA/ARS/USDFRC
Route #1
Prairie du Sac, WI 53578
(608) 643-2438
(608) 263-7775

B.C. Venuto
Management Agronomist
USDA/ARS/USDFRC
Route #1
Prairie du Sac, WI 53578
(608) 643-2438
(608) 263-7775

*R.P. Walgenbach
Research Agronomist
(608) 263-8925
FTS: 364-5372

*Madison location address is:
USDA/ARS/USDFRC
1925 Linden Drive West
University of Wisconsin
Madison, WI 53706
(608) 263-2030
FTS: 364-5240

CONTENTS

PREDICTING SOIL K SUPPLY

I.T. AIGHEWI and M.P. RUSSELLE

INTRODUCTION

Most plants require large amounts of the nutrient potassium (K) for high yield and quality. Although K fertilizer recommendations for crops are generally based on the amount we can easily extract from the soil (for example, exchangeable K), other forms and K supply indexes less suitable for routine soil testing are known. In addition, only air-dried topsoil samples are usually analyzed, and deeper samples from the subsoil are not taken. This has often led to inaccurate K fertilizer recommendations to farmers, which means either lower yields or excessive fertilizer costs.

Subsoils are frequently very difficult to sample, because of stones, wet conditions, or compacted layers. The objective of this research was to develop and verify prediction equations for several important soil K supply indexes from soil chemical and physical properties typically published in soil survey reports by the Soil Conservation Service.

MATERIALS AND METHODS

Ten Minnesota soils derived from different parent materials were sampled by genetic horizon to a depth of 1.5 m, resulting in 47 samples. Each sample was analyzed for exchangeable K, solution K, non-exchangeable K, potential K buffering capacity, and 39 other chemical and physical characteristics. Methodology typical of that used by the SCS was used for the independent characteristics. Exchangeable K was determined by extracting both air-dry and moist (-33 kPa) samples with 1 M ammonium acetate (pH 7.0), because the former is standard for topsoil testing, but air drying often greatly alters values of exchangeable K, especially in subsoils. Solution K was determined by immiscible displacement with tetrachloroethylene, non-exchangeable K was determined as "step K" by repetitive extraction with boiling 1 M HNO_3, and potential K buffering capacity was determined by standard quantity/intensity curves.

Stepwise multiple regression was used to develop empirical prediction equations for each K index. We limited the number of independent variables to a maximum of four, and replaced less typical variables with those which appear more frequently in the Soil Survey database (e.g., organic C concentration is more frequently included in the database than is the related value of organic matter, and is also generally measured with more precision).

Independent samples were obtained from eight other soil series in Minnesota to verify the applicability of the equations. Analysis of these samples was conducted as before. Data were also obtained from published soil characterization tables to verify the equation for exchangeable K on air-dry samples (the only K availability index determined by Soil Survey). Data were included from Minnesota and seven other North Central states. The

relationship between actual and predicted K was used to test the applicability of the prediction equations.

RESULTS AND DISCUSSION

Prediction equations with sufficiently high R^2 values were developed for exchangeable K (air-dry), non-exchangeable K, and potential K buffering capacity, but we were unable to achieve this result with exchangeable K (-33 kPa) and solution K. All three predictive equations had R^2 values between 89 and 92%, and included one or more of the variables: organic C concentration, sum of exchangeable bases, total silt concentration, and bulk density. These results strongly support our working hypothesis that soil K indexes can be predicted from other soil characteristics. The reason for the failure to develop equations for two indexes is unknown, and we are continuing this effort.

Not all soil characteristics have been determined at this time for the sampled validation set, therefore we cannot report how well the equations work on independent soil samples from Minnesota. However, the equation for exchangeable K (air-dry) was tested against the published Soil Survey data. Within Minnesota, on a group of 34 topsoils and subsoils, the linear relationship between predicted and actual exchangeable K was close (r^2=91%), although the slope was only 0.91 (predicted/actual). When only subsoils were considered, the slope approached 1 (0.97) and the r^2 value remained high (92%). The relationship was not as close when soils from seven other states and an independent group of Minnesota soils were tested. The slope of the relationship fell to 0.72 and the r^2 decreased to 62%. This result may have been caused by including soils of different parent material than those of the initial dataset.

We are presently examining the dataset in an attempt to discern characteristics shared by the outliers. We plan to adjust the prediction equations based on this new knowledge to allow more dependable prediction of exchangeable K (air-dry). Equations for the other K indexes will also be validated and improved, if necessary. The resulting equations could be used to construct subsoil fertility maps where adequate Soil Survey data are available and could be combined with topsoil tests to modify K fertilizer recommendations for forage and field crops.

LEGUME RESPONSE TO FRESH DAIRY COW EXCRETA

M.P. RUSSELLE and G.C. BUZICKY

INTRODUCTION

Symbiotic N_2-fixation is the major contributor to the terrestrial N cycle and can be the primary source of N in legume-based crop and livestock systems. Recycling of N by return of animal excreta to the fields occurs during pasturing or through mechanical spreading. In grazing situations, fecal pats and urine spots are often separated, except in bedding or watering

areas. The N application rate in these spots is variable, but generally high, and each source provides N of different availability to the plants. Availability of these N sources can reduce the quantity of N_2 fixed, thereby affecting the net input of newly-fixed N to the system. The objective of this research was to quantify the separate effects of dairy cow feces and urine on sources of N utilized by different legumes, and in particular to measure their effects on symbiotic N_2 fixation.

MATERIALS AND METHODS

The experiment was established on a Hubbard loamy sand in central Minnesota. The plot area was limed to pH 6.5 and fertilized according to soil test recommendations. Main plots (5.5 X 6 m with 18 cm row spacing) were seeded on May 20 to 'Blazer' alfalfa, 'Arlington' red clover, 'Norcen' birdsfoot trefoil, 'Monarch' cicer milkvetch, and 'Remount' sainfoin. Each legume was appropriately inoculated, with the exception of sainfoin, the seed of which was surface sterilized with methyl alcohol and sodium hypochlorite. No effective nodules were found on the sainfoin, making it an appropriate non-N_2-fixing control species. Three microplots (0.9 X 1.5 m) were established within each main plot about one week before planting. ^{15}N-labeled ammonium sulfate was injected to label the soil N pool. In the second year of the experiment, the ^{15}N label was applied to new microplots in the established stands in late April, before appreciable regrowth had occurred. Small applications of ammonium nitrate were made to the sainfoin to encourage growth.

In both years, all plots were first harvested when alfalfa was between one-tenth and one-half bloom (July 28 in the establishment year and June 7-8 in the production year). Either feces or urine collected from high producing Holsteins was poured between the planted rows at rates of about 10 kg total (1.3 kg dry) or 7.3 kg (7.3 L)/m^2, respectively, within one week after the first harvest each year. Untreated plots were maintained for each species. The rate of urine application was reduced to about 5.4 L/m^2 in the second year. The second herbage harvest each year was removed about six weeks after the first harvest. This plant material was rapidly oven-dried, weighed, ground, and analyzed for total N and ^{15}N content. Amounts of N derived from fixation were calculated using isotope dilution methodology. Data from the five replicates were analyzed as a split plot arrangement in a randomized complete block design, with species as main plots and excreta treatments as subplots.

RESULTS AND DISCUSSION

In both years, birdsfoot trefoil had a typical "burned" appearance after urine application, whereas this symptom was not apparent in any other species. This occurred despite a 25% reduction in urine application rate the second year. There was no yield response to excreta application in the stand establishment year. Alfalfa and red clover yielded about 2.2 Mg/ha, birdsfoot trefoil yielded 1.3 Mg/ha, and cicer milkvetch yielded 0.9 Mg/ha in the Sept harvest. Herbage yield in the following year varied with species, but there was an interaction with excreta treatment. Alfalfa (ave. 4.0 Mg/ha), red clover (ave. 4.3 Mg/ha), and birdsfoot trefoil (ave.

2.2 Mg/ha) yields were not affected by treatment, whereas cicer milkvetch yields increased from an average of 2.9 Mg/ha in control or feces treatments to 4.0 Mg/ha in the urine treatment.

The amount of symbiotically-fixed N_2 removed in herbage averaged 75 kg/ha in red clover and alfalfa, 55 kg/ha in cicer milkvetch, and only 32 kg/ha in birdsfoot trefoil (Table 1). As expected, the greatest amount of N_2 fixation had occurred in untreated plants (ave. 81 kg/ha across species), treatment with feces resulted in 15 kg/ha less N_2 fixation, and treatment with urine caused a 52 kg/ha decrease from the control. Estimated recovery of N from feces and urine ranged from only 10 and 27 kg/ha in birdsfoot trefoil to an average of 22 and 81 kg/ha in the other legumes, respectively. The sensitivity of birdsfoot trefoil to urine application may be a previously unrecognized limitation to its performance in pastures.

These measurements substantiate earlier concerns about the inefficiency of N cycling in pasture conditions, particularly with reference to N from urine. Not only did urine application decrease the extent of symbiotic N_2 fixation in all vigorously-growing legume species, but urine N was poorly utilized by the legumes. Much of the N in urine may be lost by volatilization and a substantial proportion may be leached beyond the root zone under humid or irrigated conditions.

Table 1. Amount of herbage N derived from symbiotic N_2 fixation during the first six weeks after application of fresh dairy cow excreta (mean and standard error).

Year	Species	Treatment		
		Control	Feces	Urine
		kg N/ha		
Establishment	Alfalfa	38 (1)	28 (6)	12 (8)
	Red clover	43 (6)	27 (4)	18 (6)
	Birdsfoot trefoil	22 (2)	27 (4)	8 (3)
	Cicer milkvetch	10 (4)	2 (1)	9 (7)
Production	Alfalfa	95 (6)	80 (9)	36 (9)
	Red clover	97 (7)	87 (10)	48 (10)
	Birdsfoot trefoil	53 (9)	32 (4)	12 (2)
	Cicer Milkvetch	75 (17)	65 (8)	25 (7)

MARATHON - A NEW RED CLOVER

R.R. SMITH AND D.K. SHARPEE

A new cultivar of red clover, Marathon, was jointly released by the USDA-ARS and Wisconsin Agricultural Experiment Station in May, 1987. Marathon is a winter-hardy, disease resistant cultivar providing good production through the third year (second harvest year) adopted to northern United States.

DEVELOPMENT

Marathon is an advanced generation synthetic (Syn 5) cultivar developed by using phenotypic selection for persistence in and tolerance to wet soils and for resistance to the disease northern anthracnose (NA). Forty-five clones were selected from three red clover populations in the spring of 1979 after three years of exposure to wet, acid (pH 5.7) soils on the Wis. Agric. Sta., Marshfield, WI. Progeny of the 45 polycross families were screened in the greenhouse for NA. Five resistant plants from each family were intercrossed in isolation to produce Marathon.

PERFORMANCE

Marathon persists in the field longer than other red clover, has better winterhardiness than Arlington, has good resistance to NA and moderate resistance to powdery mildew and consistently exceed Arlington in the second harvest year (Table 1). In addition, Marathon is similar to Arlington in maturity, seed yield, and neutral and acid detergent fiber concentration.

Table 1. Mean Forage Yield, Percent Stand, Disease Resistance and quality of Marathon and Arlington red clover (number of tests in paranthesis).

	Forage Yield*				% Stand #		Disease			
	1st yr.+	2nd yr.	3rd yr.	Total % Arl	2nd yr.	3rd yr.	NA++	PM	NDF*	ADF**
at Arlington)										
Marathon	4.89(6)	4.14(5)	3.06(3)	113	78	60	2.2	MR	39.6	25.2
Arlington	4.74(6)	3.61(5)	2.42(3)	100	62	25	2.5	R	38.7	26.2
Wisconsin Ave.										
Marathon	4.33(10)	3.88(9)	--	108	80	--	--	--	--	--
Arlington	4.16(10)	3.46(9)	--	100	60	--	--	--	--	--

*Tons Dry Matter Per Acre at 12% Moisture; + First year is first harvest year after seeding year; i.e. 2nd year of growth; # Percent of full stand in October; ++ NA=northern anthraconse, 1=resistant; 5=suceptible; PM= powdery mildew, R=resistant, MR=moderately resistant; ** Avg. five harvests over two years as determined by lab analysis in bud stage of growth.

GENETIC ANALYSIS OF POLLEN MORPHOLOGY OF BILATERALLY-DERIVED TETRAPLOID RED CLOVER

J.E. TOFTE AND R.R. SMITH

The most accurate method to determine chromosome numbers in plants is the cytological analysis of root tips. This method is very time consuming; therefore, numerous plant morphological characters have been used with various degrees of success to distinguish diploid (2n=2x=14) red clover plants from tetraploid (2n=4x=28) plants. Probably the most reliable morphological character is the

shape of dry pollen grains. Typically diploids produce pollen containing the x number of chromosomes (7) and are cylindrical (sausage-like) in shape. Tetraploid on the the other hand produce pollen which contains the 2x number of chromosomes (14) and this pollen is generally tetrahedral or triangular in shape. On occasion some tetraploid plants, irrespective of how they were derived, will produce cylindrical shaped pollen, typically of diploid plants. Due to micotic abnormalities some diploid plants produce functional tetrahedral shaped pollen which contain the 2x number of chromosomes (2n gamete). If this is true for diploids, then is the cylindrical shaped pollen from tetraploids x or 2x in chromsome constitution?

MATERIALS AND METHODS

Observations of the morphology of dry pollen in a bilaterally-derived (2x-2x) tetraploid population (Bi4x) indicated that 78% of the plants produced 20% or more cylindrical shaped pollen. To determine the genetic constitution (ploidy level) of the cylindrical shaped pollen several Bi4x (tetraploid) plants with a high frequency of cylindrical pollen were crossed to diploid (2x) and treploid (4x) plants.

RESULTS

No seed was obtained from 775 crosses using the diploid as the female (Table 1). This would be expected if the cylindrical shaped pollen Bi4x plants had a genetic constitution of n=2x=14 and not n=x=7, typical of cylindrical shaped pollen of diploids. Immature seed were observed on seven of the 758 2x-Bi4x crosses. The premature death of the seed probably resulted from a breakdown of the endosperm. Only one seed was obtained from 758 crosses when the tetraploid were used as females. The pollen produced on Bi4x tetraploids was functional as evidenced by the seed produced when crossed to unrelated tetraploid plants of the C21 population. These results would suggest that some 2x pollen grains from the tetraploids revert to the cylindrical shape, typical of normal diploids, and that the shape of the pollen may be under genetic control.

Table 1. Number of crosses and seed produced and fertility of bilaterally-derived tetraploid red clover plants when crossed to unrelated diploids and tetraploids.

Cross[1]	Type Cross	Number of Crosses	Number of Seed	Fertility (Seeds/Cross)
C760 x Bi4x	2x-4x	775	0	0.00
Bi4X x C760	4x-2x	758	1	<0.01
C21 x Bi4x	4x-4x	374	62	0.17
Bi4x x C21	4x-4x	381	75	0.19

[1]Female listed first; C760 are diploid; C21 are unrelated tetraploid plants.

SPRING CLIPPING ALFALFA MANAGEMENT STUDY

R.P. WALGENBACH

INTRODUCTION

Harvesting of a large number of alfalfa acres during varying weather conditions at near an optimum maturity stage often presents an obstacle to obtaining the herbage of uniform and consistent chemical composition required to meet the needs of high producing dairy cows. The length that optimum quality is available will vary from season to season with varying weather conditions but generally ranges from seven to ten days in length. Current alfalfa cultivars differ only about 2 to 3 days in their rate of first crop development in the upper Midwest. Spreading out the range of first crop alfalfa development could help producers to harvest forage that had overall higher quality with a more consistent chemical composition. The objectives of this study were to determine the influence of spring clipping and cultivars on alfalfa dry matter yield, chemical composition, and estimated milk production potential.

MATERIALS AND METHODS

'Peak', 'Epic', and '130' alfalfa's were established each year prior to initiation of spring clipping. Fertilizer was applied each season to maintain high levels of fertility. Three spring clippings were imposed and consisted of clipping to leave about a 5 cm stubble. The schedules imposed are presented in table 1. The crops after spring clippings were allowed to regrow for 45 to 50 days and then subsequent crops were harvested at between mid to late bud stages. Autumn cuts were made in 1985 and 1986 but not 1987. Residual influences from early spring clipping treatments during 1985 and 1986 were measured in first crop regrowth of the subsequent years. Plots measured 7.6 m long by 1.8 m wide and were arranged in a split plot design, with clipping dates and cutting schedules as whole plots and cultivars as subplots.

RESULTS AND DISCUSSION

The total seasonal dry matter (DM) yields and estimated milk production potential (MPP) are presented in table 2. If DM from spring clipping and autumn cuttings are included, the 1985 and 1986 DM yields of schedule B and C were near or greater than those of intensively manage alfalfa. The 1987 yields did not include autumn cutting yields and this lowered the total seasonal yields. The third crop of spring clipped alfalfa (data not presented) yielded more DM than did that of intensively managed alfalfa. This indicated that spring clipped alfalfa recovered from any adverse spring clipping effects.

First crop yields following the Spring clipped year differed little in DM yield or percent alfalfa compared to unclipped intensively cut alfalfa in 1986. But, in 1987 all spring clipped and the intensively cut alfalfa produced less first crop DM and had fewer plants compared to that and those of leniently managed alfalfa. The 1986-87 autumn and winter weather was more conducive to winter injury compared to that of 1985-86.

Autumn cutting magnified this winter injury effect. Spring clipping of well fertilized multiple pest resistant alfalfa cultivars appears to be an option to extend the availability of high quality forage during first crop harvest. But, reduced seasonal DM yields may result especially if autumn crops are not harvested.

Table 1. Approximate dates of spring clipping (SC) and subsequent cutting of alfalfa in 1985, 1986 and 1987.[1,2]

Schedule	Harvest				
	SC	1st	2nd	3rd	4th[4]
	date				
A	4-20	6-6	7-8	8-13	10-17
B	4-26	6-13	7-12	8-13	10-17
C	5-2	6-20	7-15	8-20	10-17
D		5-26	6-27	7-29	8-31
E[3]		6-9	7-17	8-27	

[1]Dates in 1987 were 5 to 7 days later than these dates.
[2]Each year cuttings were made on a year after seeding stand of alfalfa.
[3]Schedule E was initiated in 1986.
[4]No autumn cutting occurred in 1987.

Table 2. Mean first crop dry matter yield early spring alfalfa ground cover of three alfalfa cultivars clipped the previous year on three dates in early spring with subsequent crops cut near mid to late bud stages and cut using 4 time intensive and 3 time lenient schedules.

Schedule	First Crop Yield[1]		Alfalfa as percent[2] of ground cover	
	1986	1987	1986	1987
	kg/ha		%	
A	5382	4775	87	71
B	5439	4764	86	70
C	5599	5108	91	69
D	5686	4476	92	62
E	--	5719	--	88
$LSD_{.05}$	NS	787	NS	

[1]All plots harvested in late May of year following spring clipping.
[2]Visual estimates.

Table 3. Mean seasonal dry matter yield and milk production potential of three alfalfa cultivars clipped on three dates in early spring with subsequent crops cut near mid to late bud stages and cut using 4 time intensive and 3 time lenient schedules.

Schedule	Seasonal Yields[1]			Milk Production Potential[2]
	1985	1986	1987	1986
	--------kg DM/ha----------			kg milk/ha/yr
A	13725	13300	10253	12,003
B	14049	13229	10754	13,042
C	15010	13620	11748	14,806
D	14105	11639	12406	12,440
E	--	13871	12174	10,947

[1]Yields include DM from spring clipping and autumn crops in 1985 and 1986 for schedules A,B, and C. No autumn crops were cut for these schedules in 1987.

[2]Estimated using neutral detergent and acid detergent fiber to predict digestible dry matter intake for a 1513 kg cow producing 90 kg of 4.0% fat milk/day. Formula obtained from W.T. Howard, Department of Dairy Science, University of Wisconsin, Madison.

CHANGES IN THE PECTIC FRACTION OF ALFALFA STEM CELL WALLS WITH MATURATION

R.D. HATFIELD

INTRODUCTION:

As plants mature there is a continual deposition of structural polysaccharides into the cell wall matrix. The types of polysaccharides synthesized and deposited within the matrix varies with the plant species and to some extent upon the environmental conditions. Pectic polysaccharides are usually deposited early during cell wall formation primarily in the middle lamella. However, changes can occur in the degree of methyl esterification of galacturonsyl residues, molecular size of individual polymers, neutral sugar substitution, and the degree of inter-polymer interactions or cross-links. The objectives of this investigation was to characterize the pectic fraction during maturation of alfalfa cell walls.

PROCEDURE:

Alfalfa (Medicago sativa) plants were grown in a greenhouse and harvested at early bud stage. Plants were freeze dried and separated into leaves and stems. The stems were divided according to maturity into three groups, a mature group (lower 7-8 nodes), less mature (upper 7-8 nodes), and an immature group (apical 2-3 cm of main stems and branches). Starch free cell walls were prepared from each fraction and subjected to sequential extraction to remove

pectic materials. The extraction sequence consisted of four steps: 1) 1st pectin extract, 0.5% ammonium oxalate (pH 3.5 70-80°C for 1 hour); 2) 2nd pectin extract, a repeat of step one; 3) lignin extract, delignification (acidic sodium chlorite), and 4) 3rd pectin extract, 0.5% ammonium oxalate as in step one. Isolated fractions were dialyzed against distilled water and freeze dried. Each fraction was analyzed for total sugar, total uronics, and neutral sugar composition. Subsamples were subjected to DEAE anion exchange chromatography. Individual fractions were analyzed for total sugar and uronics and pooled fractions were analyzed for neutral sugar composition.

RESULTS AND DISCUSSION:

During cell wall development there was a decline in acidic polysaccharide (pectic fraction) synthesis and an increase in the neutral polysaccharides. In alfalfa this was reflected by a decrease in the proportion of pectic polysaccharides in the total cell wall matrix with increasing maturity (Table I). This decline of approximately 45% may partially be due to a natural degradative turnover of pectic polysaccharides in maturing cell wall matrices. The percent of uronic acids in each fraction remains relatively constant during maturation with the lignin fractions being lowest (41.6% to 45.9%).

To assess if changes were occurring in the individual polysaccharides that made up each isolated fraction, samples were subjected to DEAE anion exchange chromatography. Column profiles were divided into unbound and increasing tightly bound fractions (Fig. 1). The DEAE column profiles within each group of different isolates (1st pectin, 3rd pectin, or lignin) were all similar irrespective of maturity. Lignin extracts contained the greatest heterogeneity in column profiles (Fig 1). Neutral sugar composition of unbound fractions isolated by the first pectin extract were similar except for the apical region which contained approximately 33% more arabinose than the more mature regions. This suggests possible changes in the neutral polysaccharides associated with the pectic fraction. The greatest variations were seen in the neutral sugar composition of DEAE fractions obtained from lignin extracts (Fig. 2). There was an increase in xylose content of fractions 2 and 3. This may reflect an increase in the synthesis of acidic xylans that become closely associated with the pectic fraction and were co-extracted.

As the alfalfa stems increase in maturity the proportion of the total pectic material resistant to ammonia oxalate extraction increases (Table I). This would indicate increased interactions between pectic polysaccharides and lignin or other cell wall components to inhibit extraction. The changes in the neutral sugar composition seen in the isolated fractions may be important in the alterations in these interactions.

Table I. Total carbohydrate recovered from alfalfa stems subjected to pectin extraction sequence. Stem samples were isolated from regions differing in maturity.

	Grams of Carbohydrate Recovered				
	1 st pectin	2 nd pectin	legnin removal	3 rd pectin	grams/ grams CW
Apical region	0.0526 (54.42)*	0.0302 (45.20)	0.0458 (45.86)	0.0374 (50.70)	0.1764
Upper nodes	0.0464 (60.80)	0.0282 (52.14)	0.0382 (41.61)	0.026 (57.13)	0.1396
Lower nodes	0.0277 (58.71)	0.0078 (51.33)	0.0316 (43.30)	0.0293 (58.86)	0.096

* Number in parenthesis represents the % uronics of each fraction.

PECTIC POLYSACCHARIDE INTERACTIONS WITHIN ALFALFA CELL WALLS

R.D. HATFIELD

INTRODUCTION:

The pectic polysaccharides are considered to be the most easily extracted of the cell wall components. Extraction requirements are usually mild in chemical nature consisting of cold or hot water followed by treatments with metal ion chelators. These methods are usually sufficient to remove the highly esterifed polygalacturonans as well as the more tightly bound negatively charged polyalacturonans that are held in place by calcium ion cross-links. Previous observations indicated that alfalfa stems contained a pectic fraction (50%) that was recalcitrant to typical pectin extraction procedures. The resistance to extraction may be due to chemical bonding between pectic polysaccharides and other cell wall polysaccharides or lignin. The objective of this study was to begin preliminary investigations to determine the nature of the cell wall interactions that results in this resistance to extraction.

PROCEDURE:

Starch free cell walls were prepared from the lower stem nodes of alfalfa plants harvested at the early bud stage. Cell wall preparations were exhaustively extracted with 0.5% ammonium oxalate (pH 3.5, 70-80°C) to remove the readily extractable pectic polysaccharides. After thorough washing with cold distilled water the partially depectinated walls (Dp-CW) were freeze dried and stored over P_2O_5 until used. Subsamples of the DP-CW (250 mg) were subjected to various chemical extractions to disrupt cell wall interactions. These treatments included acidic sodium chlorite (70-80°C, 2h), a commercial esterase (48 h 25°C), 0.1M sodium carbonate (25 °C, 18 h with stirring), and 0.25M sodium methoxide in methanol (25°C, 24 h with stirring) followed by water extraction (70-80°C, 1h). Isolated

fractions were dialyzed against distilled water and assayed for total sugars and total uronics. After each chemical treatment the cell wall residue was extracted with 0.5% ammonium oxalate to determine if the treatment rendered additional pectic polysaccharides amenable to solubilization.

RESULTS AND DISCUSSION:

The chemical treatments were chosen to assess the possible existence of ester type bonding patterns between pectic polysaccharides and other cell wall components. The most effective treatment for removing quantities of carbohydrate was the sodium methoxide followed by hot water extraction (Table I). The least effective was the weak acetic acid treatment. Sodium methoxide has been used as a means to remove phenolic acids from plant cell walls. The UV spectrum of the methanolic extract did not reveal the presence of phenolic acids (280-315 nm) although there was a small peak at 235 nm. This treatment would effectively disrupt a wide range of ester linkages among cell wall components.

Lignin extraction removes a portion of the pectic polysaccharides and renders an additional portion amenable to ammonium oxalate extraction (Table I). This treatment would remove both ester linkages as well as other covalent linkages that might exist between specific pectic polysaccharides and lignin. Treatment of cell walls with acetic acid alone was included to evaluate the possibility that acid labile glycosidic linkages were being hydrolyzed during delignification. This treatment was not effective in solubilizing carbohydrate. The sodium carbonate solublized almost as much as the delignification process but was not as effective in rendering additional material susceptible to ammonium oxalate extraction. Esterase treatment of cell walls was ineffective in solubilizing carbohydrate. This may be due to its poor ability to hydrolyze ester linkages within the cell wall matrix particularly in relation to the phenolic acids. This enzyme could release only 10% of the total phenolic acids in bromegrass cell walls as compared to 1N NaOH extraction.

These results would indicate that covalent linkages bind a portion of the pectic polysaccharides into the cell wall matrix. The major type of covalent interaction would most likely be in the form of ester bond between components. However, it is not possible at this time to assign these linkages as occurring between different polysaccharide polymers or between polysaccharides and lignin. It is possible that direct ester linkages could be formed between galacturonsyl residues and accessible hydroxyl groups on the lignin polymer. Lignin may be bound to a small number of pectic polysaccharides with covalent linkages other than of the ester type. This is based on the observation that acidic sodium chlorite treatment following the other treatments solubilize an additional carbohydrate fraction.

Table I. Total carbohydrate released from partially depectinated alfalfa cell walls by various chemical treatments.

	Micrograms Total Cabohydrates	
Chemical	Initial Extraction	Ammonium Oxalate Extraction
Sodium Chlorite	5700 (37.34)	3300 (42.94)
Acetic Acid	------	1300 (35.88)
Sodium Carbonate	4600 (42.29)	1100 (36.24)
Esterase	1200 (50.07)	900 (14.26)
Sodium Methoxide	13130 (33.30)	1000 (44.60)

Numbers in () are the percent uronic acid in each fraction.

INHIBITION OF FERMENTATION BY SOLUBLE PHENOLIC-CARBOHYDRATE COMPLEXES

H.G. JUNG

INTRODUCTION

The phenolic material in plant cell walls is negatively associated with forage fiber fermentation. It is this limited digestibility of fiber that determines the utilization of forages by dairy cattle. Previous research has shown that ruminal fermentation of forage fiber results in the release of soluble phenolic:carbohydrate complexes. The addition of free phenolic compounds to in vitro fermentation systems has depressed cellulose and hemicellulose fermentation. The study reported here attempted to measure the concentration and composition of phenolic:carbohydrate complexes released during fermentation of forage cell walls, and determine if these complexes would inhibit fiber digestion.

MATERIALS AND METHODS

Neutral detergent fiber was prepared from alfalfa and smooth bromegrass hays, and corn silage. These fibers were fermented with rumen fluid for 48-hr and the resultant liquid phase was collected. The identities and concentrations of phenolic monomers in the soluble phenolic:carbohydrate complexes (PCC) were established by reverse-phase liquid chromatography. Replacement with these fermentation supernatants of 50 and 100% of the buffer solution used to ferment purified cellulose and hemicellulose was done to determine if the PCC inhibited digestion. A molecular sieve fractionation was also used to more clearly identify the biologically active PCC.

RESULTS AND DISCUSSION

In vitro dry matter disappearance (IVDMD) of both cellulose and hemicellulose was depressed by additions of all fermentation supernatants (Table 1).

Table 1. Digestibility of cellulose and hemicellulose in the presence of PCC from various forages.

Treatment	IVDMD (%) cellulose 50%	cellulose 100%	hemicellulose 50%	hemicellulose 100%
control	----61.8----		-----78.3----	
alfalfa hay	42.4*	28.1*	67.9*	59.3*
smooth bromegrass hay	39.3*	27.9*	68.5*	58.4*
corn silage	47.5*	33.8*	71.3*	64.5*

*Treatment mean is lower (P<.05) than the appropriate control.

Concentrations of several phenolic monomers in both core and non-core lignin fractions were negatively correlated with IVDMD. The PCC caused an increase in the acetate-:propionate ratio of fermentation endproducts for cellulose, but a decrease in the ratio was seen for hemicellulose fermentation. Inhibition of IVDMD of both substrates was caused by small, but distinct, molecular weight compounds. Only non-core lignin phenolics were responsible for fermentation inhibition. This study was unable to pin-point the identity of the inhibitory PCC and this identification is the focus of continuing research.

INFLUENCE OF ESTERIFIED NON-CORE LIGNIN ON HEMICELLULOSE FERMENTABILITY

H.G. JUNG

INTRODUCTION

The presence of esterified non-core lignin, p-coumaric and ferulic acids, in grasses has been shown to be negatively correlated with digestibility. When poor quality grass forages, such as wheat straw, are treated with alkali this non-core lignin is removed and digestibility of the straw is improved. Legumes contain much lower concentrations of non-core lignin and their digestibility is not altered by alkali treatment. These observations have generated much interest in esterified non-core lignin as a controlling factor in forage fiber digestion. The work described here is part of a continuing effort to develop model laboratory systems in which the mechanisms of fiber fermentation and its inhibition by lignins can be studied directly without reliance on correlation analysis.

MATERIALS AND METHODS

A commercially available hemicellulose preparation, oatspelt xylan, was fractionated into A-linear, B-linear and B-branched components. These fractions have progressively more substitution of the xylan backbone with other cell

wall pentoses. p-Coumaric and ferulic acids were esterified to these hemicelluloses by first derivatizing the phenolic acids with thionyl chloride and then forming ester linkages between the phenolic carboxyl groups and hydroxyl moites on the carbohydrates. Sufficient phenolic acids were reacted with the hemicelluloses to theoretically incorporate 0, 25, 50, 75 and 100g·kg^{-1} phenolic esters in the hemicellulose. However, subsequent analysis of the treated hemicellulose showed actual ester concentrations ranged from 0 to 60g·kg^{-1}. In vitro dry matter disappearance (IVDMD) of the hemicellulose was determined after 48-h fermentations with rumen fluid.

RESULTS AND DISCUSSION

The IVDMD of the treated hemicelluloses is shown in Table 1. All three hemicellulosic fractions were different for IVDMD regardless of how much non-core lignin was esterified. The A-linear fraction was the most digestible and B-linear had the lowest IVDMD. Treatment with 50g·kg^{-1} or more of phenolic acid consistently inhibited IVDMD. When hemicellulose fraction and identity of the non-core lignin phenolic were included as covariates in a regression, analysis, the partial regression coefficients indicated that ferulic acid inhibition of IVDMD was significantly greater than that for p-coumaric acid over the range of concentrations of esterified non-core lignin achieved in this study. Although this model laboratory system is not a perfect representation of nature, it does provide two improvements over previous studies in that the non-core lignin is covalently bound to hemicellulose and inhibition of IVDMD was observed at concentrations within those reported to occur in forages.

Table 1. Influence of esterified non-core lignin components on in vitro digestibility.

Hemicellulose fraction	Compound[1]	Treatment Concentration (g·kg^{-1}) 1	25	50	75	100
A-linear	PCA	54.9^{a}	51.6^{b}	52.5^{b}	44.8^{c}	54.2^{c}
	FA	53.7^{a}	52.3^{a}	47.3^{b}	42.8^{c}	42.0^{c}
B-linear	PCA	34.1^{a}	32.1^{a}	32.2^{b}	27.9^{c}	29.5^{c}
	FA	33.6^{a}	32.1^{a}	30.5^{b}	27.1^{c}	30.0^{b}
B-branched	PCA	49.0^{a}	46.8^{b}	45.5^{bc}	43.1^{d}	44.7^{cd}
	FA	50.8^{a}	43.7^{b}	44.4^{b}	40.7^{c}	43.3^{b}

[1]p-Coumaric acid (PCA); ferulic acid (FA).

^{abcd}Means in the same row not sharing a common superscript are different (P.05).

IN VITRO DIGESTION KINETICS OF TEMPERATE PERENNIAL FORAGE LEGUME AND GRASS STEMS

D.R. BUXTON

INTRODUCTION

Herbage digestibility is a function of digestion kinetics and rate of passage from the rumen. Digestibility is limited primarily by the concentration of cell wall and its degradability. Cell walls can be divided into a potentially digestible fraction (PDCW) and an indigestible fraction (IR). The IR is not digested even when fermented for a long time. Intake of herbage is influenced by both the size of the IR and by the digestion rate of the PDCW. This study was conducted to gain information on plant factors that affect in vitro kinetics of cell wall digestion. A better understanding of plant factors that influence digestion kinetics should help in developing cultivars with improved digestibility. Stems were studied because of their homogeneity and importance in limiting digestibility of herbage.

MATERIALS AND METHODS

Analyses were conducted on the basal 150 mm of immature and mature stems of field grown cultivars of alfalfa, birdsfoot trefoil, red clover, smooth bromegrass, and orchardgrass. Stem samples were digested in rumen fluid, and data were fitted with a first-order, nonlinear model to estimate PDCW, IR, digestion rate of PDCW, and digestion lag. The cell-wall neutral sugars and lignin phenolic compounds of the plant samples were determined previously for another study.

RESULTS AND DISCUSSION

The IR concentration of dry matter increased 125% with maturity in grass stems and 68% in legume stems (Table 1). In mature stems, about two-thirds of the cell wall was indigestible. Within legume and grass species, IR on a cell-wall basis was closely correlated with lignin concentration, r = 0.84 and 0.94, respectively. The IR:lignin ration was 71% larger in grass than in legume stems with values that ranged from 2.20 to 5.02. The ratio varied with species within grasses and legumes and with maturity.

To determine factors associated with IR beyond lignin concentration and those that may account for the greater apparent effect of lignin on IR in grass than in legume stems, stepwise regression was performed. This analysis included both lignin (lignin concentration, its nitrobenzene oxidation potential, and p-coumaric acid concentration) and hemicellulose (proportion rhamnose in the total neutral sugars and arabinose:xylose ratio) terms and accounted for 87% of the variation in IR.

Size of the PDCW pool on a dry-matter basis was 77% larger in immature grass than in immature legume stems (Table 1). It decreased 43% with maturity in grass stems, but only 6% in legume stems. In mature stems, differences among species were small. The digestion lag ranged from 0.6 to 5.2 h. It was

negatively related to ferulic acid concentration in cell walls of grass stems (r = -0.31) and to syringaldehyde concentration in cell walls of legume stems (r = -0.31). Digestion rate of PDCW was 50% faster in legume than in grass stems. It also was 70% faster in immature than in mature stems.

Accessibility to surface area of PDCW probably varies with cell-wall concentration. Thus, it may be inappropriate to relate digestion rate of PDCW directly to constituents of cell-wall chemistry. Therefore the digestion rate was adjusted through a surface area fermentability index (SAF), as proposed by D. S. Fisher, J. C. Burns, and K. R. Pond (unpublished manuscript), before relating to cell-wall chemistry. The SAF was calculated as (digestion rate x PDCW)/(100 - IR). The SAF values, which adjusted digestion rates for cell-wall concentration and surface area, were more closely related to digestion rate in grasses (r = 0.97) than in legumes (r = 0.80). The values were 48% larger in immature grass than in immature legume stems (Table 1). The SAF values were relatively unaffected by maturity in legume stems, but decreased by about two-thirds with maturity in grass stems. As a result, average SAF values were about twice as large in mature legume stems as in mature grass stems.

In a stepwise regression with SAF, again both lignin (syringaldehyde as a proportion of nitrobenzene oxidation products, nitrobenzene oxidation potential of lignin, and p-coumaric acid concentration in cell walls) and hemicellulose (galactose proportion in neutral sugars and arabinsoe:xylose ratio) terms were included. This relationship accounted for 46% of the variation in SAF. These results suggest that control of digestibility of cell walls is complex and is probably regulated by the intrinsic nature of the cell wall as well as physicochemical properties of individual polymers and monomers in the cell wall. A high proportion of syringaldehyde in core lignin and a high concentration of p-coumaric acid, as well as a high lignin concentration seem to limit digestion kinetics. Also, the hemicellulose sugars were more important than glucose in affecting digestion kinetics.

Table 1. Concentrations of indigestible residue (IR) and potentially digestible cell wall (PDCW) and surface area fermentability index (SAF) in stems of grass and legume species.

Species	IR		PDCW		SAF	
	Immature	Mature	Immature	Mature	Immature	Mature
	----------% of dry matter--------					
Smooth bromegrass	20.1	42.4	45.6	24.0	0.042	0.012
Orchardgrass	20.0	47.6	40.0	24.4	0.043	0.018
Grass mean	20.0	45.0	42.8	24.2	0.043	0.015
Alfalfa	32.4	51.2	25.8	23.7	0.032	0.024
birdsfoot trefoil	28.1	45.4	25.8	27.2	0.028	0.030
Red clover	14.8	34.3	18.1	26.6	0.025	0.024
Legume mean	27.1	45.5	24.2	25.7	0.029	0.024

WATER-STRESS EFFECTS ON ALFALFA FORAGE QUALITY AFTER ADJUSTMENT FOR MATURITY DIFFERENCES

R.A. HALIM, D.R. BUXTON
M.J. HATTENDORF AND R.E. CARLSON

INTRODUCTION

Perennial forage plants grown under water-deficit stress often have higher in vitro digestible dry matter (IVDDM) than those grown under well-watered conditions, provided that the stress was initiated early in growth of the herbage. Effects of water stress on crude-protein (CP) concentration of herbage, however, have been inconsistent. Water-stress effects on plant maturation of alfalfa have not received much attention. Forage quality of alfalfa declines with increasing maturity. If water stress delays maturation of alfalfa, then increases in forage quality during water stress may result entirely from maturity differences. It is necessary to know whether delayed plant maturity accounts for the increase in nutritive value of alfalfa to understand how water stress influences forage quality. The objective of this study was to determine forage quality response of alfalfa to water stress and to relate this to corresponding changes in plant maturity, phenological development, and growth.

MATERIALS AND METHODS

'Apollo II' alfalfa was grown in 100-L potometers set into the ground and protected by a movable rain-out shelter. Plants were watered either weekly or twice weekly to 112, 100, 88, 77, and 65% of field capacity during 2 years. Regrowth herbage was harvested at five weekly intervals beginning 3 weeks after the initial cut. Plants were divided into stem bases (portion of stems below and including the 6th node), stem tops, and leaves before forage quality analyses were conducted. Plant maturity, node number, and stem length were used as covariates to test linear effects of water stress on forage quality.

RESULTS AND DISCUSSION

Yields of plants grown at 65% field capacity were about half of those grown at 112% field capacity (Table 1). Significant linear effects of irrigation level were obtained with most forage-quality characteristics. Plant maturity decreased linearly with increasing water stress. Averaged over the harvests, leaf-to-stem ratio increased from 0.60 in the well-watered treatments to 0.72 in the most severely stressed treatment (Table 1). Delayed plant maturity and node number did not account fully for the increase in leaf-to-stem ratio under water stress when the covariate analysis was conducted.

Extent of IVDDM in stem bases, stem tops, and total herbage increased linearly with increasing water stress. The increase in stems was about 9%, whereas leaf IVDDM concentration was not affected by water stress. The response of the stems was primarily associated with increased plant maturity because inclusion of maturity as a covariate negated linear effects of irrigation level on IVDDM of stem components and total herbage.

There was a sharp contrast in response of stem and leaf CP concentration to water stress. In both stem bases and stem tops, CP concentration declined with increasing irrigation level. Conversely, in leaves, CP concentration was positively related to irrigation level, although essentially the effect was observed only when the plants were severely stressed. Total-herbage CP concentration was not significantly affected by water stress because of the contrasting response between leaves and stems (Table 1). The increase in CP concentration of stem bases grown under water stress was related to factors in addition to retardation in the rate of plant maturation and phenological development because CP concentration increased by 11% with increasing water stress, even after accounting for differences in plant maturity.

In all plant parts and total herbage, cell-wall concentration increased linearly with increasing irrigation level (Table 1). Except for stem tops, this was related to factors in addition to plant maturity. Concentration of lignin in the cell walls of leaves and stem bases was unaffected by water stress. In stem bases, cell-wall cellulose concentration increased while cell-wall hemicellulose concentration decreased with increasing level of irrigation. These relationships were significant even after accounting for differences in maturity and node number.

Our work confirms that increases in forage quality of alfalfa occurs when plants are grown under continuous water stress. Some of the increase in forage quality, such as IVDDM of stems and total herbage, can be accounted for largely by a retardation in plant maturation. Other characteristics, notably reduction in cell-wall concentration in all plant parts and increase in CP concentration of stem bases under water stress, are caused by factors in addition to differences in plant maturity.

Table 1. Effect of irrigation to the indicated levels on traits of alfalfa yield and quality. Maturity index is 0 to 9 with 9 the most mature as described by Kalu and Fick.

	Irrigation level, % field capacity				
Trait	65	77	88	100	112
Relative yield	54	65	81	88	100
Maturity index	2.5	3.2	3.4	3.5	3.8
Leaf-to-stem ratio	0.72	0.72	0.63	0.61	0.60
Herbage digestibility	65.0	64.6	63.9	64.2	63.3
Herbage cell-wall conc.	39.9	40.3	41.3	41.6	43.2
Herbage CP conc.	20.9	21.0	21.6	21.6	21.1

EFFECT OF ALKALI TREATMENT OF ALFALFA AND ORCHARDGRASS ON COMPOSITION AND IN SITU DRY MATTER DIGESTION

C.J. CANALE, S.M. ABRAMS AND L.D. MULLER

INTRODUCTION

Previous research with alkali treatment of an alfalfa/orchardgrass sward at mowing demonstrated a beneficial effect of treatment when fed in a complete ration to early-lactation Holstein cows. Specifically, alkali treatment stimulated increased dry matter intake and milk production, and resulted in increased digestibility of NDF and ADF, and a reduction in indigestible fiber. That experiment did not distinguish the effects of alkali treatment between alfalfa and orchardgrass, nor did it examine the effect of differing levels of alkali on the forage. The objectives of this study were to determine the effect of various levels of alkali treatment on rate and extent of digestion, and chemical composition of alfalfa and orchardgrass at two levels of maturity. Results presented here are of dry matter digestion and fiber composition.

MATERIALS AND METHODS

Three field replicates of a first-cutting alfalfa and orchardgrass were harvested in June at two levels of maturity (pre-bud/pre-head and mid-bloom/head). They were treated at harvest with five levels of sodium hydroxide (0, 2, 4, 6, and 8g NaOH/100g forage dry matter). Forages were hand-cut and hand-sprayed, sun-cured as hay, bagged and sub-sampled. Polyester bags, containing 8g of forage, were placed in rumen of two fistulated cows and incubated for 6, 12, 24, 36, 60 and 72 hours. Rate and extent of dry matter (reported here) and fiber digestion were measured, as were the concentration of NDF, ADF and lignin.

RESULTS AND DISCUSSION

Alkali treatment had no effect on rate of dry matter digestion, but did decrease the amount of indigestible dry matter in all four forages, with the greatest reduction occurring in the mature orchardgrass (table 1). Neutral detergent fiber content decreased in orchardgrass as a result of alkali treatment, while the reverse was true for alfalfa. Acid detergent fiber was reduced in mature orchardgrass and increased in mature alfalfa, while lignin in any of the forages was unaffected by treatment. The cause of the increase in fiber content in alfalfa due to alkali treatment has yet to be determined. Initial indications from this study are that previously cited benefits of alkali treatment were due to a decrease in undigestible fiber in both alfalfa and orchardgrass, but with the predominant portion of the benefit coming from orchardgrass. Further compositional studies on these samples should clarify this.

Table 1. Effect of alkali treatment on in situ dry matter digestion and fiber composition of alfalfa and orchardgrass hays.

Forage	Maturity	NaOH	UDM[a]	Rate[b]	NDF	ADF	Lignin
		(% DM)	(% DM)	(%/h)	------(% DM)-------		
Orchardgrass	Immature	0	14.1	6.34	63.2	31.0	2.85
		2	12.4	7.01	66.6	32.4	3.20
		4	11.8	6.65	62.4	32.6	3.63
		6	11.4	7.26	62.7	31.7	3.26
		8	9.8	6.88	59.8	30.7	3.64
	Mature	0	32.8	5.65	70.8	42.2	6.54
		2	30.4	5.18	69.9	42.2	6.33
		4	26.7	4.86	69.5	42.1	6.81
		6	23.6	5.27	70.0	41.4	7.17
		8	21.2	4.99	65.4	40.0	5.16
Alfalfa	Immature	0	18.0	11.82	29.6	23.6	4.93
		2	15.7	14.79	31.8	23.1	4.67
		4	15.4	9.88	35.5	24.7	5.08
		6	14.4	10.82	35.1	24.3	4.46
		8	14.2	9.82	35.5	24.3	4.47
	Mature	0	29.3	9.06	45.2	36.8	7.79
		2	28.5	9.05	48.5	37.8	8.44
		4	28.8	8.85	51.2	38.5	7.97
		6	27.9	6.44	50.2	38.9	8.80
		8	26.3	7.56	52.0	39.3	7.02

[a]Undigestible dry matter (equals dry matter remaining after 72 hours in situ digestion.
[b]Rate of dry matter digestion.

ALFALFA LOSSES AND QUALITY CHANGES DURING HAY HARVEST AND STORAGE

C.A. ROTZ AND S.M. ABRAMS

INTRODUCTION

Losses of dry matter and quality during the harvest and storage of alfalfa hay can be very large. Typical dry matter losses for the full process are 15 to 25% for hay made under good drying conditions and 35 to 100% for hay damaged by rain. Dry matter constituents which are highest in nutritive value to the animal are most susceptible to loss.

Losses are normally categorized as field and storage losses. Field losses occur from the standing crop through baling and include

respiration, rain and machine losses. Storage losses are attributed to respiration and microorganism activity on the hay during storage.

The objective of this work was to measure dry matter and quality losses in alfalfa hay production from the standing crop through storage to determine the relationship between losses and 1) rain damage, 2) machine treatment and 3) moisture content of the hay entering storage. This information will be useful in developing and validating models which predict losses as functions of harvest and storage conditions.

MATERIALS AND METHODS

A completely random experimental design was used with 15 trials repeated over a wide range of crop and environmental conditions during a two-year period, with 3 cuttings per year. Dry matter loss during field curing due to respiration and rainfall was measured from alfalfa samples dried in the field on wire-mesh trays. Hay quality was monitored with samples obtained immediately after mowing and at the end of each field curing day by randomly gathering and chopping material from the swath.

Swaths were raked with a parallel-bar rake when hay dried to a moisture content between 45 and 35% (wet basis). Hay which remained in the field more than 3 days was turned with a second raking; this was often rain damaged hay. Following raking, a frame was placed on the ground where the swath had laid. All unattached forage particles inside the frame were hand picked to measure the quantity and quality of losses.

When the alfalfa reached a moisture content of about 20% (wet basis), hay was baled with a conventional rectangular baler. Pickup loss was sampled using a frame as described for raking loss. Chamber loss was collected on tarps attached under the plunger and bale chamber of the baler. In each trial, approximately 40 bales were stacked in a barn for storage. Actual moisture contents at baling varied from 11 to 34%. Storage losses were determined as the loss in dry matter and quality constituents from samples obtained just before storage, after 30 days of storage and after 6 months of storage.

Forage quality was measured using Near Infrared Reflectance Spectroscopy (NIRS). Quality measurements were crude protein (CP), insoluble nitrogen (IN), in vitro dry matter disappearance (IVDMD), neutral detergent fiber (NDF) and lignin. Approximately 10% of the samples were randomly selected for standard laboratory analysis to provide data for calibration of the NIRS unit.

RESULTS AND DISCUSSION

When hay was cured without rain damage, field curing loss (respiration loss) ranged from -8 to 19% with an average of 3.2% (Table 1). The loss consisted primarily of constituents other than fiber and protein (nonstructural carbohydrate). Insolubility of the protein increased with time in the field. With rain damage, dry matter loss increased about 0.7% per mm of rain with an average loss of 11.2% (Table 1). There was a small loss of protein following rain damage.

Machine dry matter and quality losses were similar with the greatest loss occurring during the raking of swaths into windrows.

Raking loss was inversely related to the crop yield or area density of the swath. Average amounts of dry matter left by machine operations were 3.5% for raking, 0.8% for turning the windrow, 1.8% for the baler pickup and 1.1% from the baler chamber (Table 1). Machine losses were similar across all quality constituents so the quality of harvested hay was not effected much by the loss.

Storage dry matter losses ranged from 4.5% for dry hay to 10.9% for hay containing 25 to 34% moisture. Total NDF and lignin showed little change through storage while the quantity of digestible dry matter decreased 6 to 14% (Table 1). Total protein losses averaged 6.6% which was similar to the average dry matter loss of all hay. Protein loss was not related to moisture content of the hay; whereas, the loss of other constituents increased with increased hay moisture. Protein became less soluble throughout the storage period, particularly in the wetter hay.

Table 1. Total loss (% of initial crop yield) of dry matter and quality constituents during the harvest and storage of alfalfa hay.

Loss	Dry matter mean	Dry matter std. dev.	Digestible dry matter	Crude protein	Insoluble nitrogen	Neutral detergent fiber	Lignin
Field curing							
without rain	3.2	5.9	3.3	-4.5	-11.0	1.9	7.8
with rain	11.2	8.1	13.4	5.7	-3.5	0.7	8.0
Machine							
First rake[1]	3.5	2.9	3.7	3.8	3.8	3.1	2.8
Second rake[1]	0.8	0.3	0.8	0.8	0.9	0.8	0.8
Baler pickup[2]	1.8	0.7	1.7	1.7	1.8	1.8	1.9
Baler chamber[2]	1.1	0.3	1.1	1.3	1.4	0.8	0.7
Storage							
11-20% mc[3]	4.5	1.9	6.2	6.0	-1.3	1.8	-1.6
20-25% mc	7.9	2.0	11.8	8.8	-6.7	-1.2	-1.9
25-34% mc	10.9	2.5	13.5	7.5	-9.0	0.3	3.4
all	6.6	3.2	9.2	6.6	-4.8	0.6	-0.8

[1]Parallel-bar rake, loss expressed as percent of initial crop yield.
[2]Small rectangular baler.
[3]Moisture content entering storage. Loss is percent of material entering storage.

SIMULATION OF FIELD DRYING ALFALFA WITHIN THE SOIL-SWATH-ATMOSPHERE CONTINUUM

Y. CHEN AND C.A. ROTZ

INTRODUCTION

Hay is the major form of harvested alfalfa. Although techniques of artificially drying hay have been developed since 1920, most alfalfa hay is still naturally dried in the field rather than in barn dryers or dehydrators. The greatest challenge in hay-making is to dry alfalfa as quickly as possible in order to minimize losses and preserve forage quality. Dry matter losses can be very high when alfalfa remains in the field for a long period to dry. Discoloring and nutrient changes also occur during field curing due to excessive sunshine, rain and microbial activity.

During field drying, cut alfalfa remains in a soil-plant-atmosphere continuum. In the field environment, drying is a complicated process governed by physical and physiological principles. The major mechanisms are heat and moisture transfer. Many plant and environmental factors influence field drying, such as solar radiation, sky cloud cover, atmospheric temperature and humidity, wind speed, rainfall, dew, alfalfa variety, leaf area, leaf orientation, plant growth stage, plant resistance to moisture removal, swath density, soil temperature and soil moisture level.

Mathematical models based upon the physical and physiological principles of the soil-plant-atmosphere continuum provide useful tools to study field drying and to comprehensively understand the natural drying process. Through modeling, the relative importance and interrelationship among various factors can be quantified. Altering important and controllable factors can speed field drying and, thus, improve hay quality. For instance, mechanical conditioning and chemical treatments can reduce the plant's resistance to moisture removal. Tedding can manipulate the swath structure and expose undried alfalfa to direct sunlight.

The objective of this study was to develop and validate a simulation model of the soil-swath-atmosphere continuum for field drying alfalfa. Emphasis was on the dynamic behavior of the heat and moisture status within the soil-swath-atmosphere continuum, and the application of engineering principles to biological systems. The model was to be general enough to represent field drying of alfalfa under a wide range of conditions.

MATERIALS AND METHODS

A model (ALFDRY) was developed to closely simulate the field curing process. The model was based upon the physical and physiological principles governing heat and moisture transfer in the soil-swath-atmosphere continuum. Shortwave and long-wave radiation, wind speed, soil temperature, exchanges between leaf surfaces and surrounding air, between air spaces, and between the soil surface, the swath and the ambient air were modeled and integrated via energy and moisture conservation. The Monteith-Penman equation was

adapted to characterize exchanges at leaf surfaces with consideration of the equilibrium relative humidity of leaves under severe water stress. Leaf internal resistance to moisture removal (r) was modeled through an exponential function of the leaf moisture content (M) and initial moisture content (M_o):

$$r = 10^{a-bM/M_o} \qquad (1)$$

Parameters a and b were statistically determined with the use of field drying data.

Actual field drying measurements were collected over a wide range of swath densities (0.262 to 2.548 kg dry matter/m²), initial moisture contents (2.645 to 4.495 kg water/kg dry matter), maturity levels (0 to 100% bloom) and meteorological conditions for East Lansing, Michigan (1984) and Fresno, California (1985). Field data was used both to determine values for model parameters and to validate the full model. After the model was shown to be valid, a sensitivity analysis was performed to determine those parameters which had the greatest effect on alfalfa drying.

RESULTS AND DISCUSSION

The simulation model (ALFDRY) was developed from published relationships of heat and mass transfer. Reasonable values for model parameters were obtained from the literature or from measurements made during the field experiments. Derivative-free optimization and nonlinear regression were used to determine the two coefficients (a and b of eq. 1) which related leaf surface resistance to the moisture content of the swath. Coefficients were determined which provided the best fit of the model to experimental data. When averaged over all experimental conditions, an average value for a was 4.52 and b was 1.62.

Validation of the model was done by comparing simulated and actual data for swath moisture content over time for data not used in parameter estimation. Statistical comparison showed a close correlation of the two data sets with an adjusted coefficient of determination of 0.91 for actual data collected at the two locations with the large variation in moisture content and swath density.

The sensitivity analysis revealed that the model was most sensitive to changes in parameter a. Other important parameters included parameter b, leaf area index, swath density, leaf moisture content at full hydration and a conditioning factor. Alfalfa drying can be enhanced most effectively by altering these parameters.

QUICK-DRYING FORAGE MATS

R.G. KOEGEL, K.J. SHINNERS AND R.J. STRAUB

Mats made from alfalfa shredded at time of mowing and placed on stubble have been shown in earlier research to dry to a moisture content suitable for baling in 2-1/2 to 6 hours under favorable conditions. Furthermore, alfalfa so harvested proved to have a more rapid and extensive NDF digestion than conventionally harvested material.

The research prototype machine for making forage mats which was described in the 1986 research summaries was further developed and evaluated. Major modifications included a completely new mat-forming press designed to reduce complexity, weight, and friction; an alternative macerator unit to be evaluated relative to the original unit; and aids to obtaining mat uniformity.

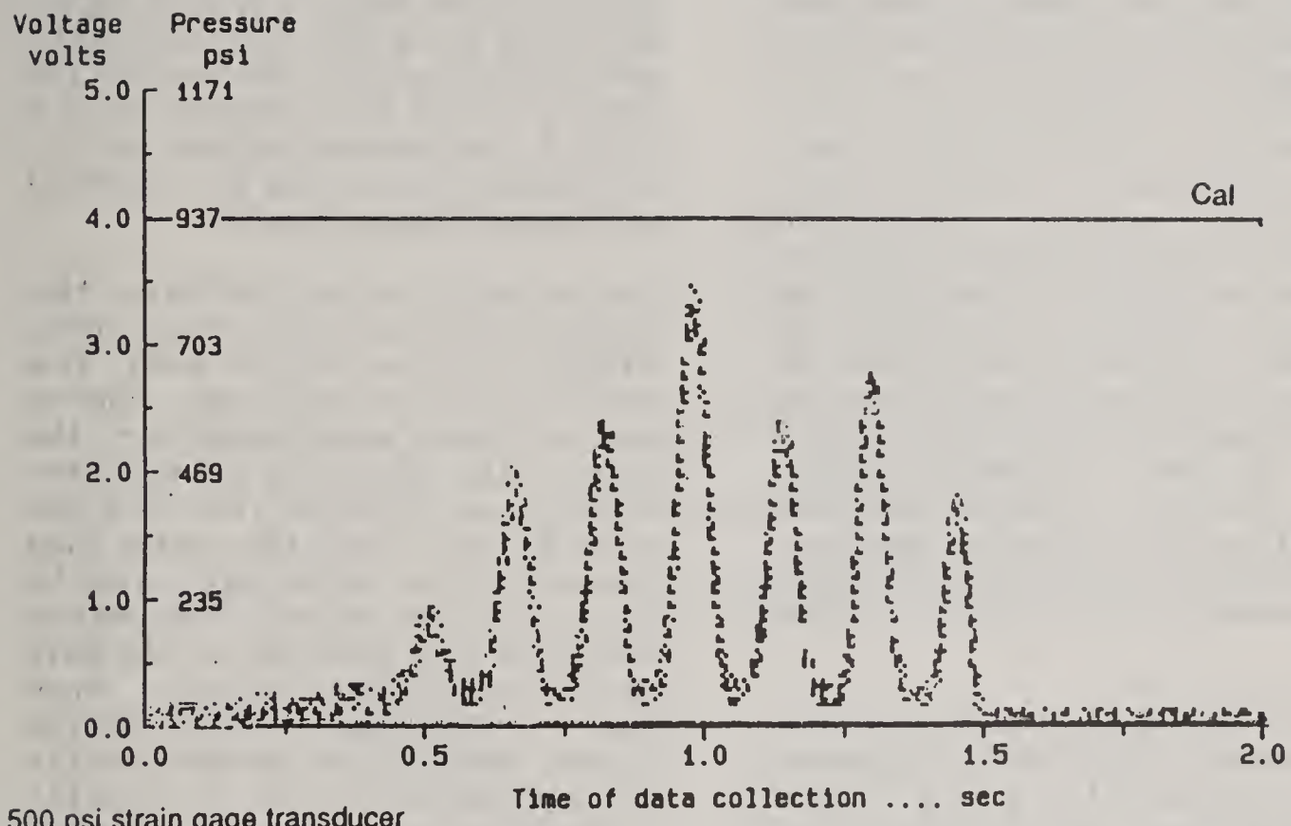
The mat forming press was designed in such a way that pressure could be applied to the forage by (1) tension in the press belt wrapping around the drum, (2) force on one to three pneumatic rolls applied to the press belt, or (3) various combinations of (1) and (2). The pneumatic rolls could, in addition, be pressurized to different levels to change their characteristics.

The alternative macerator configuration functions satisfactorily. However, since it was used for research at East Lansing during the summer of 1987 (see Rotz summary), side-by-side comparisons of throughput, power requirement, and degree of processing relative to the original macerator have not yet been made.

Two types of mat uniformity were improved by interposing a spinning rotor between the macerator outlet and the press inlet: (1) distribution of coarse and fine particles within the mat and (2) distribution of the flow of forage material over the press belt. Continuous measurement of pressure during the pressing cycle was the subject of an M.S. Thesis (Close, 1987). Three different measuring techniques were used: (1) a miniature strain gauge pressure transducer, (2) a flattened fluid-filled tube connected to a large, external pressure transducer, and (3) pressure sensitive film. A computer controlled data acquisition system recorded the data. Results indicated that that pressure was applied to the forage as a series of discrete pressure spikes rather than continuously (Fig 1). This was caused by the small diameter, recirculating rollers which transmitted force from the stationary side of the press to the rotating side. When the pressures measured by the two types of transducers were compared with the pressures measured by the pressure sensitive film and with pressures calculated from free body diagrams, it became evident that transducers gave values higher than the true values. This anomaly was caused by the finite thickness and relative inelasticity of the transducers causing them to carry a load which normally would have been spread over a much larger area of forage. Coefficients were developed to convert the transducer readings to actual pressure values. The pressure histories of material passing through the press were useful in the press redesign.

Consolidation and compaction characteristics of macerated alfalfa were studied to determine how this material would pack into a silo. Results indicated that under static loading, as would be the case in a tower silo, macerated material consolidated more rapidly than conventional. However, after sufficiently long time, both came to approximately the same density. Under dynamic loading conditions, as would be the case in a horizontal silo packed by driving over it with a vehicle, macerated material reached a greater density.

Following visits to the USDFRC at Madison, three European universities and one manufacturer have initiated work on fast drying forage mats.



500 psi strain gage transducer
Test date: 9/16/86
Actuator pressure: 30 psi (0.21 MPa)
Alfalfa mat: medium load, even distibution
71% wet basis moisture content

Average pressure: 204 psi (1.41 MPa)
Maximum pressure: 820 psi (5.65 MPa)

Figure 1. Pressure trace from mat forming press.

IMPROVING THE QUALITY OF FORAGE HARVESTED IN LARGE PACKAGES

S.L. BRANDEMUEHL, R.G. KOEGEL, F.J. FRONCZAK, K.J. SHINNERS AND R.J. STRAUB

I. Finish Drying of Large, Round Bales

A study was performed to determine the feasibility and characteristics of radially drying high moisture (25-40% wb) round hay bales with ambient air to safe storage levels within 48 hours. The bales were formed with an axial, cylindrical void into which air was forced to remove the moisture radially from the bale. This is in contrast to the more conventional method of setting solid bales over air ducts and attempting to dry them with axial airflow. The major objectives for this project included evaluating the drying parameters, quality of the hay dried, labor requirements, and overall system cost.

Eighty-five bales were dried over two summers with three different fans, the majority being formed with openings for radial drying while the rest were solid for axial drying as comparisons. Many parameters were monitored and analyzed throughout the drying trials. The bales were suspended and weighed throughout the trials to determine their rate of water loss. Likewise, the progression of the drying fronts through the bales was monitored during drying. The moisture contents of the bales were also determined both initially and after drying by means of core samples, and the condition of the air entering the bales was monitored throughout the trials.

Preliminary results (Fig 2) indicate that the feasibility of radial drying within a given time period depends primarily on the bale density and the initial moisture content of the material combined with the rate of airflow through the bale. To dry bales within 48 hours to a storage moisture content of 17%, the dry matter density should be no greater than 8 lb/ft^3, the maximum initial moisture content 35%, and the airflow per bale at least 600 CFM.

The results also indicate the drying advantage of the cored, radially dried bales over the solid, axially dried ones. Due to more uniformly distributed air, the radial bales generally dried faster at the same airflows than did the axial bales. Also, the radial flow process dried bales more evenly than did the axial flow which overdried some portions of the bale while underdrying others. Such factors would make radial drying appear more attractive for practical applications since it results in reduced drying energy requirements and higher quality hay. However, many additional factors must be considered including forage type and quality, equipment and labor requirements, and the overall system cost.

II. Silage from Large Round Bales

Large round bales at approximately 65% moisture content (w.b.) were covered with plastic in three different ways to exclude air: (1) stacked bales under a single plastic cover, (2) individually wrapped in multiple layers of thin plastic strip ("Silawrap"/Underhaug), and (3) individually bagged.

The experience of this year as well

as last year points to the absolute necessity of controlling rodents since any perforation of covers led to total loss of the contents.

The wrapped bales were opened after approximately four months. Appearance and smell were good except for small areas of mold in the area of the last wrap of each bale, indicating the desirability of a few additional wraps to insure sealing.

The bale stack with a single high quality plastic cover has not yet been opened. However, monitoring of the stack temperature indicates good preservation.

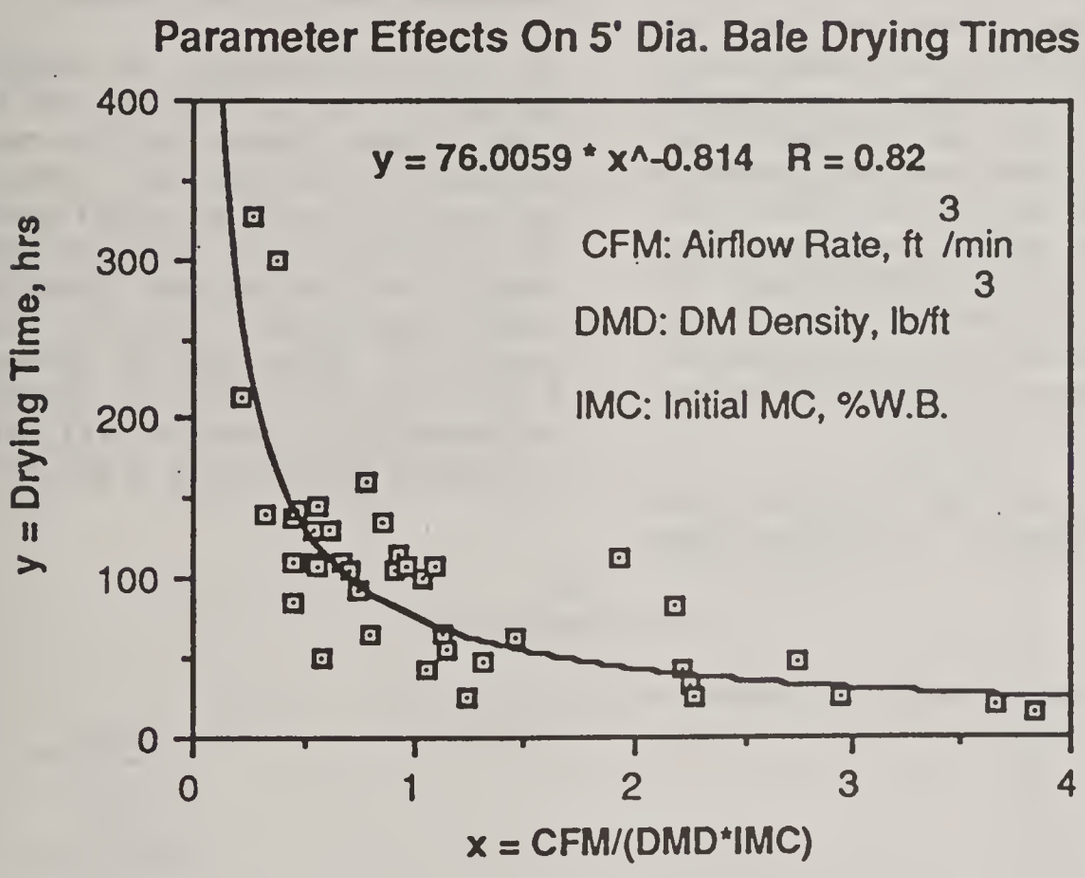


Figure 2. Bale drying time as a function of airflow rate, dry matter density, and initial moisture content.

REDUCTION IN THE ENERGY REQUIREMENT OF FORAGE HARVESTERS

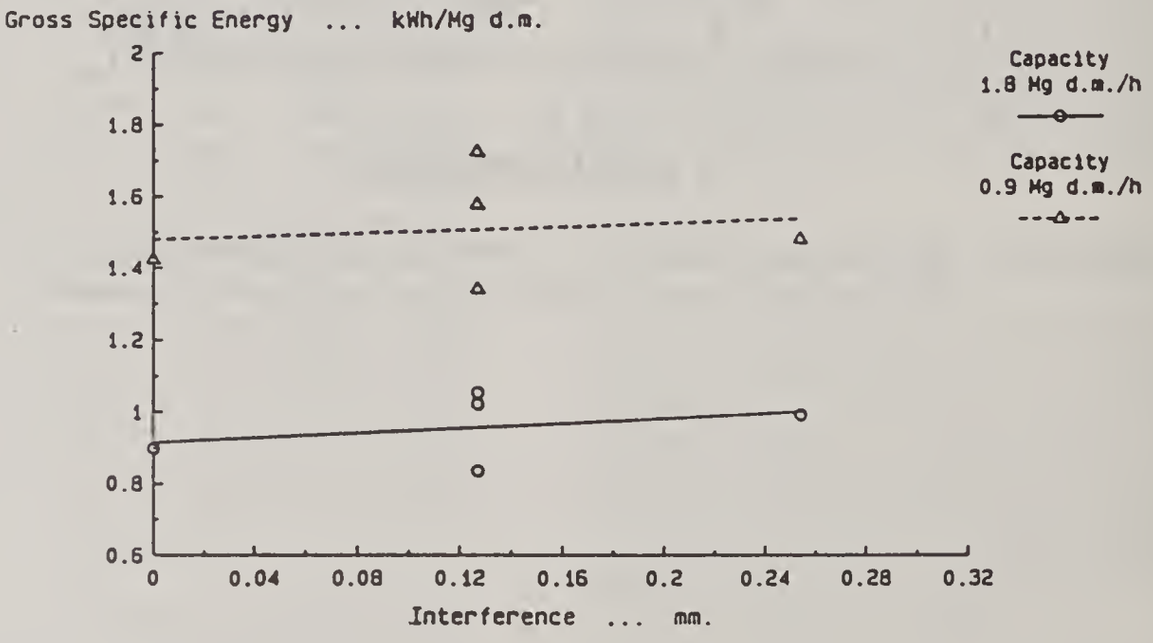
R.G. KOEGEL, F.J. FRONCZAK, K.J. SHINNERS AND R.J. STRAUB

Reduction in the specific energy required for forage chopping, in addition to reducing both operating and capital costs, could lead to more timely completion of harvesting with a resulting improvement in quality.

I. Cutterhead. Previous work has shown that significant reduction of the cutterhead energy requirement is possible. Two non-conventional cutterheads have been evaluated to date. Both appear to have advantages in energy requirements. The more recent cutterhead is described as "radial blade and anvil". Some of its characteristics are shown graphically below (Figs 3 and 4).

II. Conveying of Forage from Chopper to Wagon. The conveying function currently takes almost as much energy as chopping itself. Pneumatic conveying, as carried out on forage harvesters, generally has an efficiency of less than 10%. Mechanical conveying devices have the potential of reducing forage harvester energy requirements.

In either pneumatic or mechanical conveying the coefficient of friction between forage and the machine surfaces is an important factor in determining energy requirements. The coefficient of friction between forage and teflon was found to be approximately half that for forage and steel while that of forage and high density polyethylene was intermediate. Some initial results are shown below (Figs 5 and 6).



Equal length blades and anvils.

Figure 3. Gross specific energy vs. interference

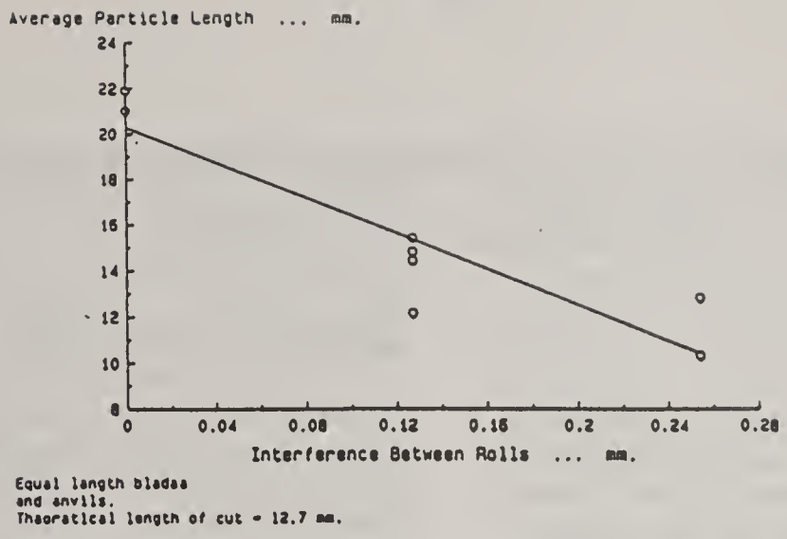


Figure 4. Average particle length vs. Interference

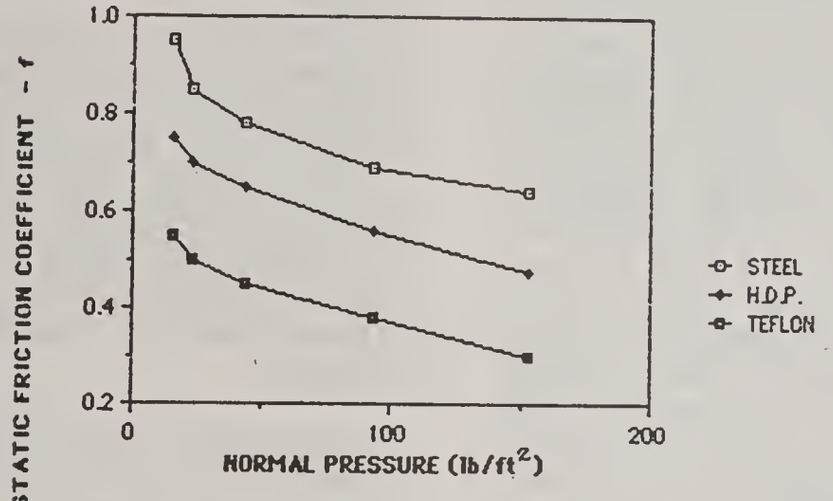


Figure 5. Static friction coefficient 70.8% (w.b.)

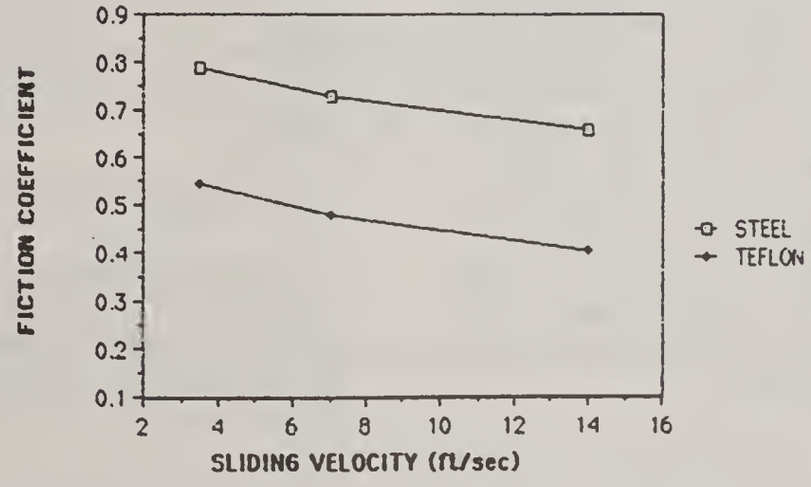


Figure 6. Sliding friction coefficient 68.6% (w.b.) Normal pressures from 38.2 to 122.2 lb/ft

PREDICTION OF LACTIC ACID BACTERIAL NUMBERS ON ALFALFA

R.E. MUCK AND P.L. O'CONNOR

INTRODUCTION

The success of bacterial inoculants in making high quality alfalfa silage has been shown to be related to the natural or epiphytic population of lactic acid bacteria (LAB). The higher the epiphytic level the lower the probability that an inoculant will be beneficial. Previous work at the research farm indicated that epiphytic populations of LAB on chopped alfalfa were related to a number of factors: wilting time, average wilting temperature, moisture content at harvest and air temperature at harvest. Regression equations developed from 1985 and 1986 data for wilting times of 3 days or less explained much of the variation in the results. The predicted values were within 1 order of magnitude of the actual values greater than 95% of the time. The only conditions not predicted well were first loads of the day, which were underpredicted, and loads where the moisture content was less than 40% w.b., which were overpredicted.

These regression equations needed to be validated on an independent set of data. Consequently, the objectives of this study were to collect LAB counts on chopped alfalfa over the 1987 harvesting season at the research farm, validate the regression equations with these data and develop a computer model to predict LAB numbers over a wider range of conditions than covered by the regression equations.

MATERIALS AND METHODS

Alternate wagon loads of alfalfa were sampled as they were emptied into silos at the research farm at Prairee du Sac. These samples were analyzed for LAB and moisture content. Weather, equipment utilized in harvesting, length of wilting, dry matter yields and swath dimensions were recorded for each cutting from each field.

Prediction of the 1987 data via the regression equations required wilting time and average wilting temperature to determine the appropriate equation. Depending on the circumstances, the independent variable in each equation was either moisture content or air temperature in the 2 h preceding harvest.

The computer model consisted of simulating two processes: LAB growth in the swath and drying of the alfalfa. LAB growth was simulated using the published equations for LAB growth used in our silage fermentation model with some modifications. Drying was simulated assuming a constant exponential decline in moisture content during typical drying hours with the exception of rainfall events.

RESULTS AND DISCUSSION

The 1987 harvesting season was much more adverse than the previous 2 seasons. Approximately half of the first harvest alfalfa received rain during wilting, and some

fields were rained on during wilting in second and third cutting. In the previous 2 seasons, wilting alfalfa rarely received precipitation. Consequently, the 1987 data provided a considerable challenge to both predictions methods.

Prediction of the 1987 data using the regression equations is shown in Figure 1. The predicted-to-observed ratio (0.962;s.e., 0.017) was statistically ($P<0.05$) less than 1. The standard error of the predicted estimate was 0.825 log units, which was 50% higher than that reported for the original data set. As in 1985-86, dry samples (>60% DM) were overpredicted. Rainfall during wilting, however, had no apparent effect on LAB numbers. Given the considerable differences in the weather conditions between 1987 and the previous years, the regression equations predicted LAB numbers well.

The computer model did not predict LAB numbers as well as the regression equations. The wetter alfalfa samples (<50% DM) were underpredicted (Pred:Obs, 0.923), and the drier ones were overpredicted (1.077). The standard errors of the estimates were also higher for computer model predictions, 0.976 and 0.920 log units, respectively. Therefore, improvements in the computer model are needed to attain the accuracy and precision of the regression equations.

Overall, it appears that prediction of LAB numbers, based on data that would be available from a farmer, is possible. Improvement of the computer model and testing of both prediction methods on a variety of farms in range of locations is necessary before making them available to farmers.

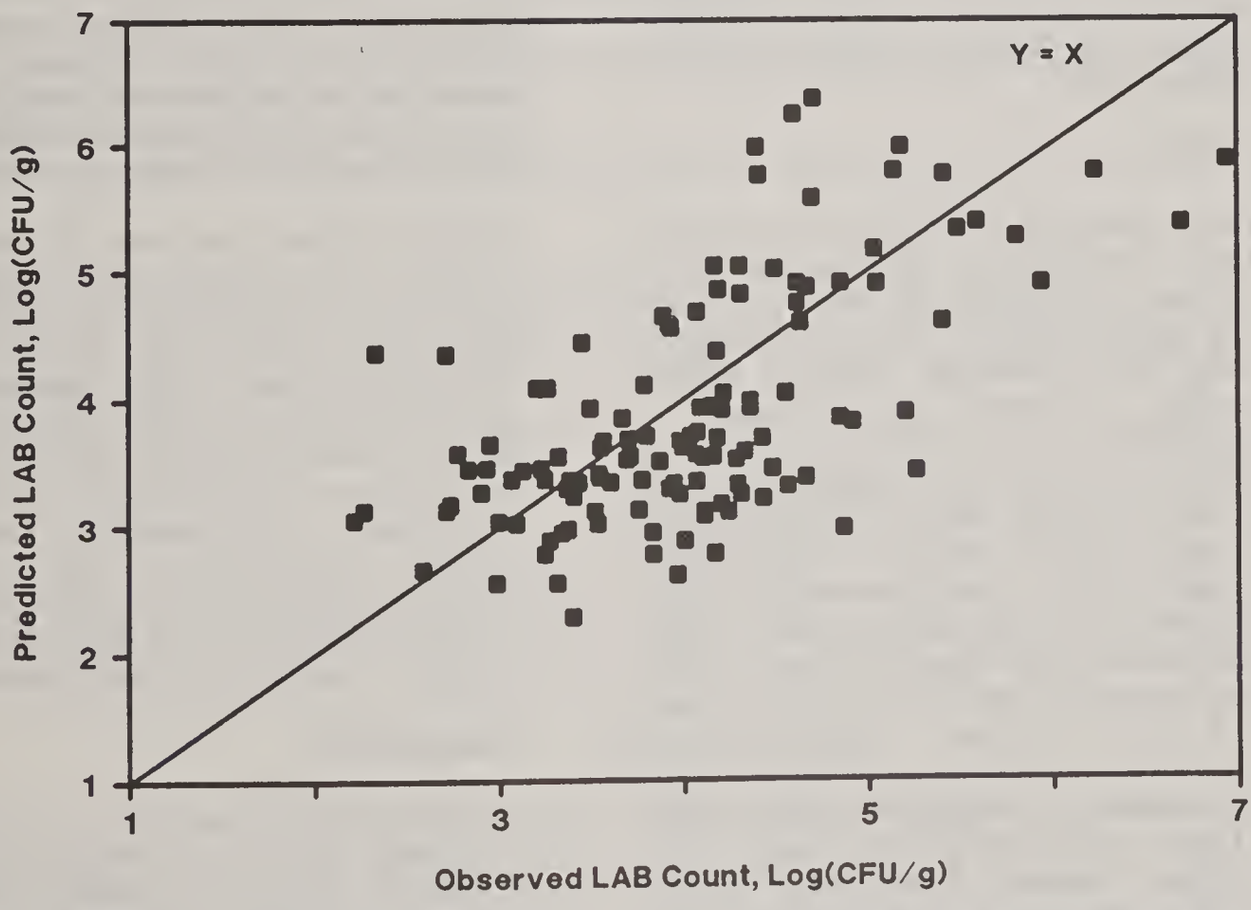


Figure 1. Prediction of 1987 data via regression equations from 1985-86 data.

EFFECT OF PLANT FACTORS, BACTERIAL INOCULANT AND SUGAR ADDITION ON FERMENTATION OF ALFALFA SILAGE

B.A. JONES, L.D. SATTER AND R.E. MUCK

INTRODUCTION

The quality of alfalfa silage is heavily influenced by the fermentation which preserves the forage. A number of factors can influence the outcome of the fermentation, including plant composition, number and kinds of bacteria present on forage tissue at ensiling time, and the supply of sugar (energy) for the bacteria. The objective of this study was to determine whether bacteria or sugar is the most likely bottleneck to quality silage making, and what effect silage moisture content has on the relative importance of these two factors. Also, the effect of cutting (first, second, third or fourth crop) on response to bacteria or sugar addition was determined.

METHODS

Four cuttings of alfalfa in each of two growing seasons were field wilted to 30-35, 40-45 or 50-55% dry matter (DM). Four treatments were applied within each dry matter group: 1. Control (C), 2. Sugar - 1 gram/50 grams alfalfa on as is basis (S), 3. Inoculum, 1,000,000 Colony Forming Units (CFU)/50 grams alfalfa (I), and 4. Inoculum + Sugar (IS). The treated alfalfa was ensiled in laboratory silos incubated at 30°C. Duplicate silos were opened and analyzed on days 1,2,3,7,14 and 60.

The number of bacteria on the plant tissue at time of ensiling fluctuated between 100 and 100,000 CFU/gram field chopped herbage. The ratio of total sugar:buffering capacity (mg sugar/g dry matter:meq/kg) of fresh forage ranged from .05 to .24. The highest ratio was observed with 4th cutting alfalfa obtained in October. Forage characteristics as influenced by season and cutting are summarized in Table 1.

All inoculated silages lowered pH faster than control or sugar treatments, with the greatest response occurring when there were less than 1000 CFU/gram of the untreated or control alfalfa. Sugar addition reduced final pH to a greater extent 71% of the time with the two wettest silages, but was without effect with the driest silage.

The effect of sugar or inoculant addition on silage chemical composition at 60 days of ensiling is shown for the 30% dry matter and 50% dry matter silages in Table 2.

Table 3 shows the overall average effect of silage treatments on nitrogen fractions in alfalfa silage. Inoculation was more effective than sugar addition in reducing NH_3 and NPN formation. Values for the medium moisture silage were intermediate and are not shown. The overall effect was small, since only two control silages (one each in the two wettest silages) had NH_3-N values greater than 10% of total N.

CONCLUSIONS

(1) The sugar content of fourth cutting alfalfa harvested in mid-October is significantly higher than in other cuttings; (2) The

buffer capacity of alfalfa tended to be lower in second and third cuttings than other cuttings; (3) Inoculation reduced pH more rapidly in all silages; (4) Silages containing 30 and 40% dry matter showed significant benefit from inoculation and sugar addition for all silage measurements. Drier silage benefited from inoculation but not sugar addition; (5) Alfalfa cuttings differ considerably in their ensiling characteristics. Plant characteristics of buffer capacity, sugar level, number of lactic acid bacteria and the ratio of sugar to buffer capacity explain most of the differences in ensiling characteristics.

Table 1. Forage characteristics as influenced by season and cutting.

Measurement	1985				1986			
	1	2	3	4	1	2	3	4
Buffering capacity[1] meq/kg DM	515^{b}	431^{d}	478^{cd}	503^{b}	580^{a}	414^{d}	524^{b}	487^{bc}
Sugars, mg/g DM[2]	49^{cd}	26^{e}	[illegible]^{c}	103^{a}	42^{d}	48^{cd}	59^{b}	106^{a}
SBC[4]	.095^{b}	.061^{c}	.112^{b}	.205^{a}	.073^{c}	.116^{b}	.114^{b}	.218^{a}
Log LAB[3]	4.4^{a}	4.2^{ab}	4.7^{a}	4.3^{ab}	3.0^{c}	4.7^{a}	4.2^{ab}	2.6^{c}

[1]meq of HCl required to drop the pH from 6 to 4.
[2]sugars extracted by 80% ethanol.
[3]LAB = epiphytic lactic acid bacteria (CFU/g), counted on Rogosa plates incubated anaerobically for 48 hrs.
[4]sugar:buffer capacity ratio.
[a-e]unlike superscripts in rows are significantly different at P<.05.

Table 2. Average value of silage measurements for 60 day silages.

Measurement	1985				1986			
	C	S	I	IS	C	S	I	IS
				30% dry matter				
pH	4.4	4.2	4.3	4.1	4.4	4.1	4.2	4.0
Lactate, % DM	8.8	10.2	9.8	10.3	9.1	10.7	9.9	11.2
Acetate, % DM	1.8	1.6	1.1	0.8	2.3	2.0	1.1	0.8
Lactate/Acetate	5.3	6.7	8.7	13.6	4.8	6.0	10.7	14.5
				50% dry matter				
pH	4.7	4.6	4.4	4.3	4.9	4.8	4.4	4.3
Lactate, % DM	5.7	6.0	6.8	6.9	3.8	4.2	6.9	6.8
Acetate, % DM	1.1	0.9	0.6	0.5	0.9	1.0	0.6	0.5
Lactate/Acetate	5.7	6.1	11.9	13.1	4.6	4.5	13.4	14.8

Table 3. Average effect of silage treatments on nitrogen fractions in alfalfa silage[1].

Treatment	NH_3	NPN	NH_3	NPN
	30-35% DM		50-55% DM	
S	83.9±13.3%	95.4±6.0%*	98.0±7.0%	96.3±6.5%*
I	68.3±13.2%	92.5±4.3%	69.8±14.7%	95.6±4.3%
IS	51.7±15.7%	86.2±6.1%	65.3±17.8%	89.1±8.3%

[1]Percent of the control silage.
*Not significant from control at P<.05.

USE OF BACTERIAL INOCULANTS TO IMPROVE ALFALFA SILAGE AND ANIMAL PERFORMANCE

J.A. WOODFORD, C.M. WACEK AND L.D. SATTER

INTRODUCTION

A series of studies was conducted to determine the influence of silage additions, mainly bacterial inoculants, on the fermentation of alfalfa silage, and its utilization by lactating cows and dairy heifers. The objective of these studies was to measure the benefit, if any, to animals consuming silage treated with a silage additive.

MATERIALS AND METHODS

All treatments were inoculants, except for two. One contained an enzyme for breaking down cellulose and the other contained 7% of a high sugar corn (as is basis) to enhance fermentation. Experiment 7 (Table 1) was a heifer growth study, while all others were lactation studies with cows beyond peak lactation. Ten studies were conducted as continuous (Experiments 1-4), simple reversal (Experiments 5,6,7 and 9), or Latin square designs (Experiments 8 and 10). Bunker silos, conventional stave silos, and oxygen limiting silos were used as storage structures.

RESULTS AND CONCLUSIONS

Silage pH (Table 1) on day 2 and 30 averaged 5.14 and 4.48 for control silages, and 4.84 and 4.30 for all treated silages. Total dry matter recovery from silos did not differ between control (93.0%) or treated (92.7%) silage. Dry matter intake was improved (P<.10) by additives in only one study, as was milk production. Milk fat percentage was unchanged except for a reduction in Experiment 8. When measured, body weight of lactating cows consuming treated silage was significantly greater (P≤.05) than control cows in three out of seven studies.

The experiments are arranged in Table 2 according to the ratio of lactic acid bacteria (LAB) in the inoculated silage:control silage.

In order to get a response in animal performance the inoculation had to increase the number of lactic acid bacteria in the treated silage by a factor of 10 or more. This means that if the forage material already had a large number of naturally occurring lactic acid bacteria, or if the inoculant did not contain enough lactic acid bacteria that were viable, the number of lactic acid bacteria in the silage would not be increased enough to make a difference. If animal response is averaged for studies where the number of lactic acid bacteria were successfully increased tenfold or more by the inoculant, then a $3 return for every $1 invested in inoculant was realized. If inoculants are used indiscriminantly, then the return drops to about $1.30 for every $1 invested. These calculations ignore the potential benefit of improved body condition that may occur with inoculated silage.

In summary, inoculants rather consistently improved alfalfa silage as evidenced by a more rapid drop in pH, slightly lower final pH, and a higher lactic acid/acetic acid ratio in the silage. Dry matter recovery was unaffected by inoculants. Milk production in any given experiment was seldom statistically significant, but a group of studies does indicate greater milk production, body weight gain and dry matter intake in lactating cows when the ratio of lactic acid bacteria in treated vs. control silage is greater than ten.

Table 1 Effect of bacterial inoculants on silage characteristics and animal performance.

Treatment	Lactic acid bacteria g^{-1} fresh alfalfa	pH 2d	pH 30d	Lactate[a] Percent of DM	Acetate[a] Percent of DM	Total Dry Matter Recovery,%	Silage %	DM Cows/ Trt	DMI[e], kg	Milk, kg	Fat, %	ADG[f], kg
1.Control	$4.0X10^4$	5.80	5.35	.85	.41	95.4	57.3	10	19.7	27.5^x	3.43	--
Chr Hansen (BIOMAX-SI)	$4.5X10^5$	5.67	4.68	3.01	.15	95.4	54.8	10	21.1	29.7^y	3.59	--
Miles (AGMASTER)	$5.6X10^6$	5.68_b	4.75	3.07	.15	93.4	56.6	10	20.2	29.2^y	3.43	--
2.Control	$5.0X10^5$	5.54_b	4.67	1.96	.56	87.3	55.7	10	23.4	26.7	3.81	--
(BIOMAX-SI)	$4.0X10^6$	5.51_b	4.44	3.13	.54	100.0	52.7	10	23.1	26.1	3.72	--
(AGMASTER)	$6.0X10^5$	4.47_b	4.37	3.69	.49	98.9	53.8	10	24.0	26.7	3.78	--
3.Control	--	--	4.50	3.81	.93	94.4	43.7	10	26.4	29.2	3.65	.24
PIONEER(1177)	$1.9X10^{4c}$	--	4.53	4.19	1.09	92.3	42.9	10	25.1	28.8	3.50	.25
4.Control	$6.60X10^{4c}$	--	4.64	3.11	.70	92.5	46.7	15	23.7	26.9	3.65	.33
PIONEER(1177)	$6.36X10^4$	--	4.47	4.54	.93	92.8	47.6	15	24.5	26.5	3.75	$.28^x$
5.Control	$4.34X10^4$	4.44	4.15	5.38	3.27	90.2	34.0	24	21.8	29.8	3.32	$.03^x$
Sugary Corn & MEDIPHARM	$2.93X10^5$	4.04	4.04	6.12	1.67	90.8	33.2	24	21.7^y	30.0	3.32	$.32^y$
6.Control	$6.28X10^3$	4.71	4.30	5.55	2.60	92.5	31.6	10	20.0^x	27.5	3.82	$.22^x$
Finnsugar Enzyme & MEDIPHARM	$9.47X10^5$	4.28	3.97	7.85	1.18	89.4	31.2	10	21.7^y	27.9	3.76	$.49^y$
7.Control	$9.13X10^2$	4.96	4.00	6.23	1.40	94.0	35.2	43^d	9.2			.90
Biotechniques (BIOPOWER)	$2.91X10^5$	4.46	3.88	7.16	1.08	93.8	34.8	44^d	9.5			.96
8.Control	$4.15X10^3$	5.71	4.25	9.07	1.94	91.5	27.3	12	19.9	26.7	3.75^x	.39
Finnsugar ENZYME	$4.15X10^3$	5.75	4.20	8.47	1.67	86.9	36.8	12	20.5	27.1	3.62^y	.41
GREAT LAKES	$7.42X10^4$	5.25	4.24	9.95	1.61	84.5	25.6	12	20.3	27.3	3.76^x	.58
MEDIPHARM	$4.55X10^5$	4.48	4.09	9.23	1.26	91.1	26.8	12	21.1	27.5	3.73^x	.60
9.Control	$2.30X10^3$	4.93	4.49	7.06	2.70	99.0	35.2	20	23.0	30.0	3.77	$.11^x$
Biotechniques (BIOPOWER)	$1.05X10^5$	4.43	4.31	7.89	1.99	95.4	34.8	20	22.1	30.0	3.78	$.19^y$
10.Control	$1.01X10^4$	5.07	4.43	--	--	--	36.3	12	19.7	27.2	3.52	-.06
Finnsugar & Great Lakes	$5.66X10^4$	4.70	4.24	--	--	--	37.2	12	19.8	27.1	3.51	.19
Great Lakes	$4.36X10^4$	4.58	4.30	--	--	--	35.8	12	19.4	26.8	3.55	.20
Qualitech	$3.67X10^5$	4.44	4.21	--	--	--	36.1	12	19.4	27.3	3.48	.23

[a]Means for feedout period except expt. 8, which is measured after 30 days of ensiling.
[b]day 3
[c]Repesents the number applied.
[d]Growing heifers
[e]DMI = Dry matter Intake per day
[f]ADG = Average Daily Gain
[xy]Means within columns by experiment differ significantly ($P<.10$).

Table 2. When to Expect a Response in Milk Production from Silage Inoculation

Ratio of LAB (Treated/Control)	Response (Treated as % of Control)	
151	101.5	
140	106.2	
110	103.0	
		Ave = 103.0
46	100.0	
36	100.4	
18	102.2	
11	108.0	
8	97.8	
7	100.7	
6	99.6	
		Ave = 99.2
4	98.5	
1	100.0	
1	98.5	

BACTERIAL INOCULUM FOR PRESERVATION OF ALFALFA HAY

C.A. ROTZ, R.J. DAVIS, D.R. BUCKMASTER AND J.W. THOMAS

INTRODUCTION

Alfalfa hay is normally baled at a moisture content of less than 20% (wet basis) to obtain stable storage. Two incentives exist for baling at a greater moisture content: 1) reduced leaf loss during harvest and 2) reduced field curing time. A desirable strategy for making alfalfa hay is one in which the crop is baled at a moisture content sufficient to minimize field losses and then properly preserved to minimize storage losses.

Bacterial inoculums are promoted and sold as a substance for improving the preservation of high moisture hay. Bacteria are sprayed on hay as it is baled. As the bacteria multiply and grow during the early stages of storage, they produce products claimed to enhance preservation.

The objective of this research was to compare inoculated, untreated dry, untreated wet and propionic acid treated wet alfalfa hay to determine the effectiveness of bacterial inoculation in improving preservation. Measurements of temperature, dry matter loss, forage quality and visual appearance of hay during storage were compared over a wide range in hay moisture. Several forms of inoculum were evaluated including: 1) lactobacillus, 2) lactobacillus plus a propionic acid producing

bacteria, 3) lactobacillus plus enzyme additives and 4) nonviable lactobacillus.

MATERIALS AND METHODS

A laboratory experiment was done to evaluate mold development on hay samples in plastic bags. Two inoculum treatments were compared to untreated hay and propionic acid treated hay at 20, 25 and 30% moisture content. Inoculums were 1) Lactobacillus plantarum and Pedicoccus cerevisiae and 2) the same plus Propionibacterium shermanii. Bags of each treatment at each moisture level were sealed and laid on a lab bench at room temperature (70°F) for 45 days. The bags were examined at weekly intervals and given a score for the extent of mold present.

Six field trials were conducted over a 4 year period to evaluate lactobacillus inoculation of relatively pure alfalfa hay. The type and number of treatments varied among the 6 trials. Lactobacillus inoculum (Lactobacillus plantarum and Pedicoccus cerevisiae) was evaluated in trials 1 through 3 at one moisture level in each trial. A lactobacillus inoculum with enzyme additives (Lactobacillus plantarum, Lactobacillus brevis, Pedicoccus acidolactici, Streptococcus cremoris, Streptococcus diacetylactis plus protease, amylase and gumase) was used in trials 4, 5 and 6. In these trials, treatments were included at two levels of hay moisture content. All trials included untreated and propionic acid treated hay baled at approximately the same moisture content as inoculated hay as well as dry (<20% moisture) untreated hay.

Hay of each treatment was baled at a given moisture content and taken to a barn for storage. Ten-bale stacks of each treatment were stored for a 45 day period. Hay temperature was recorded during storage and dry matter loss over the storage period was measured. Heat development in the hay was calculated as the number of degree-days in which hay temperature was above ambient temperature during the first 30 days of storage. After storage, hay was given a visual score for color and moldiness and samples were evaluated for forage quality.

RESULTS AND DISCUSSION

Inoculation of 20 to 30% moisture hay sealed in plastic bags did not alter the development or growth of mold. Similar scores were given to both inoculated and untreated samples at a given moisture content. After 28 days, mold scores were actually greater for the inoculated hay than for untreated hay. Hay treated with 1% propionic acid had no visible mold at any moisture level.

In the field experiment, inoculation of high-moisture alfalfa hay did not provide effective preservation. Heat development during the first 30 days of storage was not reduced by inoculation in any trial. Propionic acid treated hay had lower storage temperatures in 6 of 7 comparisons to inoculated hay. The exception was a trial where hay moisture content for all treatments was less than 20%.

Dry matter losses during storage were related to temperature, i.e., greater losses occurred where storage temperatures were high. In

no case did inoculation of hay reduce dry matter loss.

Hay which heated in storage had a greater content of acid detergent insoluble protein following storage. Since dry matter loss during storage consists of forage contents other than fiber, hay with the greatest loss had the greatest increase in ADF during storage. Since inoculation had no effect on heating and loss, no effect was found on forage quality.

Inoculation had some effect on the visual appearance of hay. An interaction was found between hay treatment and hay moisture content. At low moisture contents, inoculated hay had more mold and discoloration than untreated hay.

Treatment of hay baled at 22% moisture with nonviable lactobacillus bacteria caused delayed heating during storage with little effect on storage loss, quality or visual appearance when compared to inoculated and untreated hay. The nonviable bacteria may have stimulated bacterial growth which caused heating later in the storage period.

CONCLUSION

Inoculation of alfalfa hay at 20 to 40% moisture content with any of the bacterial types tested did not provide improvement in the preservation of the hay.

EFFECTS OF KCl ON FERMENTATION OF ALFALFA SILAGE

W.L. SHOCKEY AND A.L. BARTA

INTRODUCTION

Rapid attainment of low forage pH is essential for preservation of crops as silage. If plant buffering capacity were limited, a small amount of lactic acid would be sufficient to reduce the pH rapidly and to an acceptable level. Previously, researchers recognized that K, Mg, and Ca salts of organic acids could play a major role in buffer systems and indicated that the presence of inorganic ions could increase the theoretical carbohydrate requirement for fermentation of alfalfa silage. Results of recently conducted greenhouse experiments indicated that as the rate of pH decline of alfalfa silage increased alkaline mineral concentration also increased, although unmeasured components of the plant tissue may have been involved. This experiment was conducted to determine effects of potassium added as the chloride salt on the resulting fermentation of alfalfa silage.

MATERIALS AND METHODS

High quality fourth cutting alfalfa was cut, wilted (ca. 50% DM), and chopped (2-3 cm). About 200 kg of forage was well mixed by hand then divided in half. One half was untreated, the other half received KCl at a rate of 38.1 g/kg wet forage. Approximately 3 kg wet forage was then packed tightly by hand into 20 (10/treatment group) 60 x 10 cm PVC pipe sections and sealed. Two samples of each treatment were taken as pipes were filled and on days 1, 4, 7, 28, and 60 two pipes from each treatment

group were opened and the entire contents used for microbial and chemical analysis.

RESULTS AND DISCUSSION

Proximate analysis of the forage is shown in Table 1 and illustrates the high quality of the forage. Number of total anaerobes was lower for treated compared to control forage between 1 and 28 days of fermentation (Table 2). Consequently, pH declined more rapidly and to a lower level in control compared to KCl treated forage. Lactic and acetic acid production was also more rapid in the control compared to the treated silage and the negligible quantities of butyric acid in both silages at day 60 is indicative of the overall good quality of the fermentation obtained with the PVC pipes.

Although pH declined more rapidly and to a lower level in control compared to treated forage the rate of proteolysis as estimated by ammonia nitrogen concentration was also more rapid and continued to a greater extent. This observation provides evidence that rate of pH change and acid production are not necessarily the most important factors to consider when attempting to control proteolysis. Interactions with forage quality, forage dry matter content, and microbial activity both in terms of quantity and quality are also important factors to be considered.

Table 1. Proximate analysis of green herbage[1]

	Control	KCl
% Dry matter	48.5	54.1
% Crude protein	23.9	24.4
% Neutral detergent fiber	33.4	32.2
% Acid detergent fiber	26.3	25.0
% Potassium	2.74	5.98
% Calcium	1.10	1.07
% Magnesium	.25	.25
% Phosphorus	.31	.30

[1]All parameters except dry matter on dry matter basis

Table 2. Fermentation characteristics of alfalfa ensiled with KCl.

Day	Anaerobes[1]	pH	% Lactic[2]	% Acetic[2]	% Butyric[2]	Ammonia-N[3]
			CONTROL			
0	10.3	6.05	0	.24	.27	8.9
1	72.5	6.03	1.65	.16	.23	11.6
4	1700.0	5.57	3.83	.63	.25	14.9
7	4000.0	5.19	5.51	.89	.19	15.1
28	1350.0	4.75	8.45	1.54	.16	20.5
60	87.5	4.69	6.75	1.92	.17	21.4
			KCL			
0	3.8	6.01	0	.1	.28	8.2
1	9.0	5.99	.98	.14	.28	10.1
4	35.0	5.97	1.18	.18	.28	11.8
7	72.5	5.86	2.03	.19	.31	13.6
28	65.0	5.28	2.88	.85	.20	14.6
60	26.0	5.09	3.89	1.07	.17	17.4

[1]x 10-6/g wet forage
[2]Dry mater basis
[3]As % total N

STORAGE TEMPERATURE EFFECTS ON PROTEOLYSIS IN ALFALFA SILAGE

R.E. MUCK AND J.T. DICKERSON

INTRODUCTION

During fermentation in the silo, the composition of the nitrogen fraction in alfalfa can change dramatically. Proteins may be hydrolyzed by plant enzymes to peptides and free amino acids that may be further acted upon by microorganisms and/or plant enzymes, producing ammonia and amines. After ensiling these non-protein nitrogen (NPN) fractions may account for 40 to 85% of the total nitrogen. This loss of true protein negatively affects the nitrogen value of the silage when developing dairy cattle rations based on the latest NRC recommendations.

Storage temperature is one factor that may affect the amount of proteolysis. Increasing temperature increases protease activity within the normal temperature ranges found in silages. However, increased temperature would also increase silage fermentation rate, reducing proteolysis. Consequently, the objective of this study was to confirm that storage temperature significantly affects proteolysis in alfalfa silages.

MATERIALS AND METHODS

The effect of temperature on proteolysis in alfalfa silage was measured using mini-silos and

alfalfa harvested with normal field equipment in first (June 3) and third (August 20) cuttings. Two dry matter levels (40 and 55% DM) were investigated in each trial. In the first trial, 3 separate areas of a field were harvested at each DM level, and 9 silos were filled with alfalfa from each area. These silos were split among three water baths maintained at 15, 25 and 35°C, respectively. Thus, there were nine silos for each temperature and DM level. In the second trial, only one area of the field was harvested. Twenty-seven silos were filled at each DM level and split among the three water baths. In both trials, the silos were incubated for 40 days and then frozen until ready for analysis.

The alfalfa ensiled was analyzed for moisture content, lactic acid bacterial numbers, buffering capacity, total Kjeldahl nitrogen (TKN), NPN, free amino acids and ammonia (NH_3). The silages were analyzed for moisture content, TKN, NPN, free amino acids, NH_3, pH and fermentation endproducts.

RESULTS AND DISCUSSION

The pH's and fermentation endproducts of all the silages indicated that all of the alfalfa underwent a good fermentation and were well-preserved. As indicated in Table 1, increasing temperature consistently increased proteolysis as indicated by NPN level although the increase between 25 and 35°C was not always statistically significant ($P>0.10$). A 20°C change in storage temperature resulted in approximately a 10 percentage unit change in the amount of proteolysis at both 40 and 55% DM in both trials.

The effects of NH_3 formation were not as consistent between trials (Table 2). There were significant increases in NH_3 level from increasing storage temperature from 15 to 25°C but not from 25 to 35°C. NH_3 formation at both DM's was much less in the second trial, and DM level appeared to have a more significant effect on NH_3 level than did storage temperature.

The implications of this study are that temperature does play a significant role in the degree of proteolysis occurring in the silo. From a practical standpoint, air temperature at harvest, the quickness of filling, the completeness of silo sealing and the heat absorption properties of the silo will all affect silage temperature and consequently affect the amount of alfalfa nitrogen which remains as true protein through ensiling.

Table 1. NPN as a percent of TKN in the ensiled alfalfa.

Trial	DM Level	Temperature		
		15°C	25°C	35°C
1	40%	52.0	58.9	61.3
2	40	48.5	56.8	59.5
1	55%	46.4	50.9	58.5
2	55	44.1	55.7	54.2

Table 2. NH_3 as a percent of TKN in the ensiled alfalfa.

Trial	DM Level	Temperature 15°C	25°C	35°C
1	40%	9.3	10.5	9.0
2	40	4.1	5.2	5.1
1	55%	4.2	7.5	8.8
2	55	2.4	2.7	2.7

PRODUCTION OF COWS IN EARLY LACTATION FED TALLOW OR YELLOW GREASE WITH ALFALFA SILAGE OR HAY-BASED DIETS

W.L. SHOCKEY AND D.L. PALMQUIST

INTRODUCTION

High producing dairy cows, especially in early lactation, cannot consume enough energy to meet the demands of lactation. Feeding fat, a higher density form of energy than carbohydrate, is an effective way to supply additional energy without requiring an increase in total dry matter intake.

Generally, highly saturated fats are considered to be superior to unsaturated fats or oils for lactating dairy cows. Reports from extension agents and private feed consultants suggest that fat supplementation of high moisture or silage-based diets may not be as effective as fat supplementation of hay-based diets.

An experiment was conducted to compare the effectiveness of fat supplementation as either tallow (saturated) or yellow grease (unsaturated) when fed with either a high moisture alfalfa silage-based diet or a lower moisture alfalfa hay-based diet.

MATERIALS AND METHODS

Four groups of five cows in early lactation (average day of lactation at start of experiment = 54) were fed total mixed rations consisting of corn silage: concentrate:alfalfa in a 20:40:40 ratio on a dry matter basis. Alfalfa was fed as silage or chopped hay to provide diet dry matters of 50% or 67%, respectively. Cows received .7 kg/day of tallow or yellow grease. Dry matter intake, milk yield, milk fat, milk protein, and fat-corrected milk yield were measured.

RESULTS AND DISCUSSION

Responses to diets containing alfalfa silage-tallow, alfalfa silage-yellow grease, alfalfa hay-tallow, alfalfa hay-yellow grease are shown in the table. Dry matter intake, milk fat %, and fat-corrected milk yield were higher for cows fed alfalfa silage

compared to those fed alfalfa hay. Cows fed hay-yellow grease produced less fat-corrected milk compared to all other diets.

The results support the generally accepted concept that saturated fats are superior to unsaturated fats for lactating dairy cows. However, under our conditions, there was no evidence to suggest that feeding fat with an alfalfa silage-based diet was inferior to feeding fat with alfalfa fed as dry chopped hay. In fact, our results indicated superior performance for cows fed fat supplemented alfalfa silage-based diets compared to fat supplemented alfalfa hay-based diets.

Performance of lactating dairy cows fed alfalfa silage or alfalfa hay-based diets with tallow or yellow grease.

	Alfalfa Silage		Alfalfa Hay	
	Tallow	Yellow Grease	Tallow	Yellow Grease
Dry matter intake kg/day	23.4	22.8	21.5	20.7
Milk yield kg/day	30.5	29.4	31.2	29.9
Milk fat %	3.68	3.57	3.06	2.87
Milk protein %	3.12	3.04	3.01	3.04
4% Fat-corrected milk yield kg/day	28.7	27.6	27.8	24.7

EFFECT OF DIETARY FORAGE:GRAIN RATIO ON PERFORMANCE OF LACTATING DAIRY COWS

N.J. TESSMANN, H.D. RADLOFF, T.R. DHIMAN, J. KLEINMANS AND L.D. SATTER

INTRODUCTION

The purpose of this study was to measure the "diminishing returns" of increased grain in the dairy ration when high quality alfalfa silage is the forage. This study was done with first calf heifers (primiparous) and with cows (multiparous).

METHODS AND PROCEDURES

Forty-nine primiparous and 44 multiparous Holsteins were assigned to 5 dietary forage:grain ratios (trts. 1-5) at parturition. The percentage of alfalfa silage in the ration was fixed and changed from early (wks 1-12), mid (wks 13-26) and late lactation (wks 27-44)

regardless of production level (Table 1). High moisture ear corn, soybean meal, and a vitamin-mineral supplement made up the concentrate mix and along with alfalfa silage was fed in total mixed rations (TMR's) once daily.

Daily feed intake and milk production was measured. A weekly composite of am:pm milk samples was analyzed for fat %, protein % and somatic cell count by Wisconsin Dairy Herd Improvement Association (DHI). Body weights were recorded once weekly and body condition scores determined at the end of lactation. Health and reproductive data were also recorded. DHI 305d projection factors and equations were used to extend milk, fat and protein production for records short of 305d.

RESULTS AND CONCLUSIONS

Milk production for trts 1, 2 and 3 were similar for primiparous cows, and trts 1 and 2 were similar for multiparous cows (Table 2, Figs. 1&2). Cheese yield tended to be higher for multiparous cows in trt 2 compared to trt 1, possibly due to a slight milkfat test depression on the high grain diet of trt 1 (Fig. 3). This did not seem to be a problem with the primiparous cows (Fig 4). Milk production for trt 5 was significantly different from all other trts in primiparous cows and significantly different from trts 1-3 but not 4 in multiparous cows. Lactation curves are shown in Figures 5-6.

An interesting observation is the depression of milk production and percent milk protein (especially in primiparous cows) with the high forage diets. Although they were fed a good quality forage high in protein, this data suggests the possibility that protein as well as energy may be limiting milk production when high forage diets are fed. Further research in this area is underway.

DIETARY TREATMENTS

TREATMENT	ALFALFA SILAGE IN RATION IN LACTATION WEEKS 1-12	13-26	27-44
	%		
1	38.2	48.2	68.2
2	48.2	58.2	78.2
3	58.2	68.2	88.2
4	68.2	88.2	98.2
5	98.2	98.2	98.2

Table 1.

% Forage 1-12	13-26	27-44	n Primi	Multi	305d Milk,kg Primi	Multi	305d CY[1],kg Primi	Multi	305d FCM[2]kg Primi	Multi	Ser/Con[3] Primi	Multi	Ave. DOP[4] Primi	Multi
38.2	48.2	68.2	11	8	7243^{a}	8641^{a}	735^{a}	826^{ab}	7334^{a}	8295^{ab}	3.0	1.7	107	94
48.2	58.2	78.2	10	10	7091^{a}	8315^{ab}	695^{a}	852^{a}	7177^{a}	8659^{a}	1.8	2.8	79	118
58.2	68.2	88.2	11	8	6819^{a}	7453^{bc}	673^{a}	735^{bc}	6976^{a}	7563^{bc}	2.0	2.6	108	99
68.2	88.2	98.2	9	9	6005^{b}	6666^{cd}	569^{b}	663^{cd}	6014^{b}	6849^{cd}	2.1	1.6	89	73
98.2	98.2	98.2	8	9	4609^{c}	5768^{d}	448^{c}	575^{d}	4750^{c}	6000^{d}	2.0	2.3	96	124

^{abcd}Means in same column differ (P<.05); [1]Cheese yield; [2]3.5% FCM; [3]Services/Conception; [4]Days open.

Table 2.

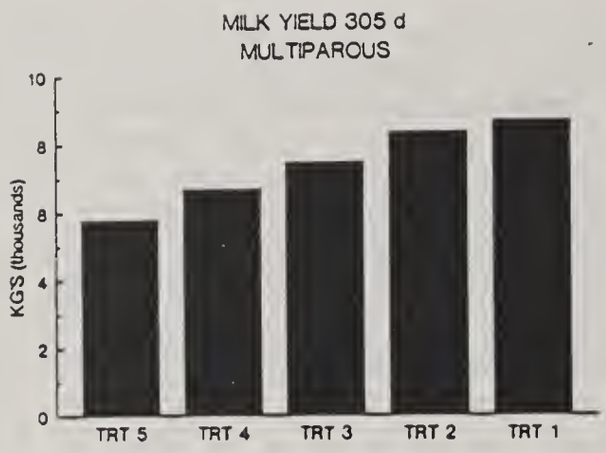


Figure 1

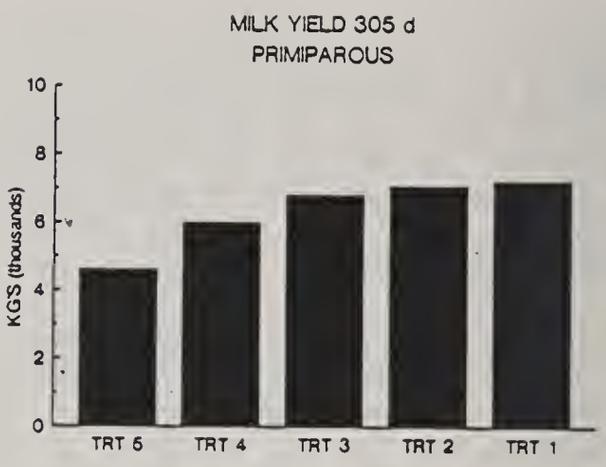


Figure 2

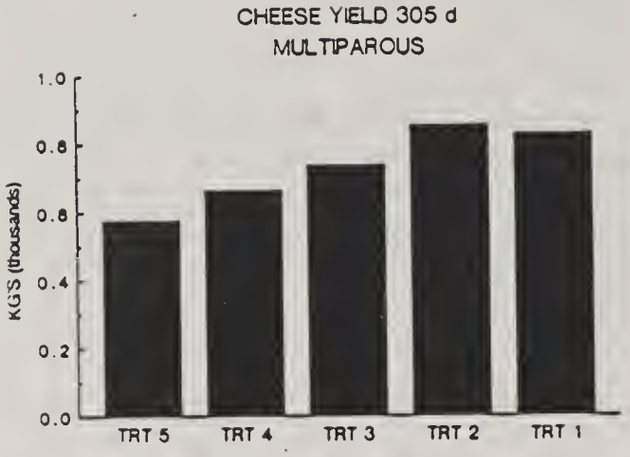


Figure 3

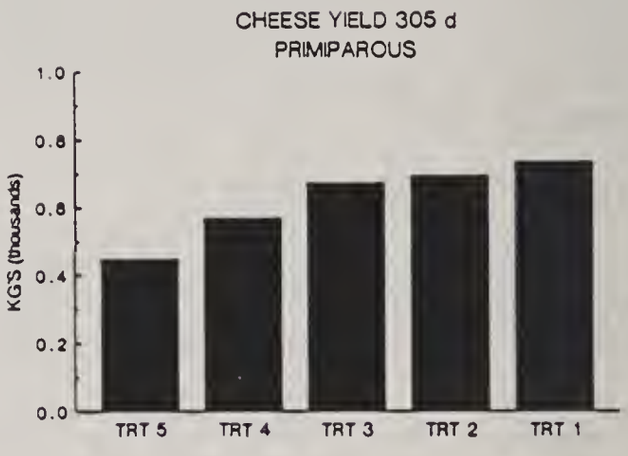


Figure 4

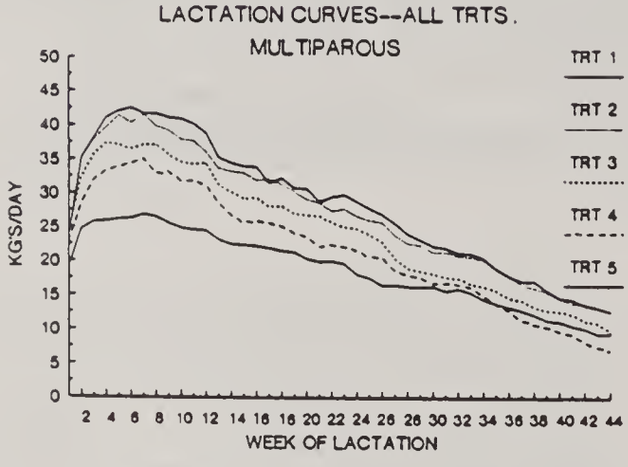


Figure 5

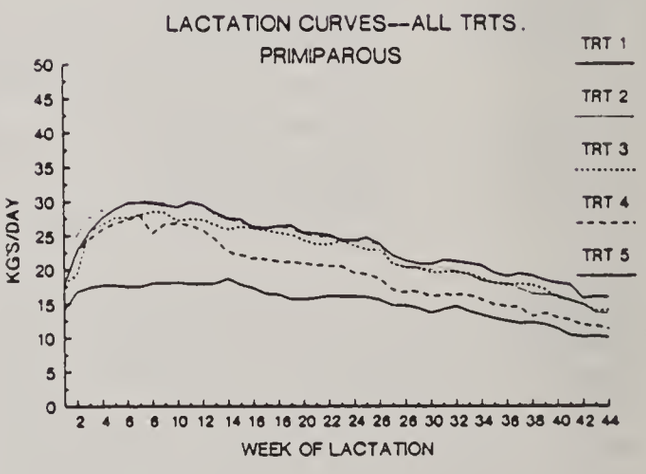


Figure 6

EFFECT OF DIETARY FORAGE:GRAIN RATIO ON RESPONSE OF LACTATING DAIRY COWS TO RECOMBINANT BOVINE SOMATOTROPIN

N.J. TESSMANN, J. KLEINMANS, T.R. DHIMAN, H.D. RADLOFF AND L.D. SATTER

INTRODUCTION

The purpose of this study was to measure the effectiveness of recombinant bovine somatotropin (rBST) when administered to first calf heifers (primiparous) or to cows (multiparous) receiving either a high energy (high grain) or a moderately low energy (high forage) diet.

METHOD AND PROCEDURES

Thirty-two primiparous and 32 multiparous Holsteins were assigned to 2 dietary forage:grain ratios (trt 1,3) at time of parturition to provide 38.2% or 58.2% alfalfa silage (DM basis) in early lactation (wks 1-12), 48.2 or 68.2 in mid lactation (wks 13-26), and 68.2 or 88.2 in late lactation (wks 27-44) (Table 1). Animals were further divided into 2 groups in each trt to receive daily injections of either saline (placebo) or 20.6 mg/hd/d of rBST (American Cyanamid Co.) from wks 13-43 (or until the second to last week of trial).

Total mixed rations (TMR's) of alfalfa silage, HMEC, SBM, and a vitamin-mineral mix were fed 1x daily. Forage:grain ratios were changed at the beginning of week 13 and week 27 of lactation, independent of production. Daily milk weights were recorded. A weekly composite of am:pm milk samples were analyzed for fat %, protein % and somatic cell count by Wisconsin Dairy Herd Improvement Association (DHI). Body weights were recorded once weekly and body condition scores done at the end of lactation. Health and reproductive data were also recorded. DHI 305d projection factors and equations were used to extend milk, fat and protein production for records short of 305d.

RESULTS AND CONCLUSIONS

There were no significant differences between placebo and rBST groups in milk, fat and protein yield in the first twelve weeks of lactation before initiation of treatment injection (within diet treatments and age groups). Milk production (305d) did increase by 19.9 and 18.3% for multiparous cows on trts 1 and 3 and 13.0 and 5.9% for primiparous cows in trts 1 and 3 (table 2, figs. 1&2). Lactation curves for each group are shown in figures 3-6.

rBST was more effective in increasing milk production in cows than in heifers. There were no effects of rBST on milk composition or on animal health and reproductive performance. Body condition scores at the end of lactation averaged $3.3\pm.95$ and $2.9\pm.69$ for multiparous cows on trt 1 PLB and trt 1 rBST, $3.5\pm.93$ and $2.3\pm.95$ for multiparous cows on trt 3 PLB and trt 3 rBST. For primiparous cows they were $3.7\pm.82$ and 3.0 ± 0 for trt 1 PLB and trt 1 rBST, and $3.5\pm.84$ and $2.7\pm.52$ for trt 3 PLB and trt 3 rBST. This suggests the dry cow diets for cows previously treated with rBST, especially for cows fed high forage diets during lactation, may need to have higher energy content to prepare the cow for the subsequent lactation.

DIETARY TREATMENTS

TREATMENT	ALFALFA SILAGE IN RATION IN LACTATION WEEKS 1-12	13-26	27-44
	----------%		----------
1 PLACEBO	38.2	48.2	68.2
1 BST	38.2	48.2	68.2
3 PLACEBO	58.2	68.2	88.2
3 BST	58.2	68.2	88.2

Table 1

Trt.	n		305 d Milk		305d CY[1]		305d FMC[2]		Serv/Conception		Ave. DOP[3]	
	Primi	Multi	Primi	Multi	Primi	Multi	Primi	Multi	Primi	Multi	Primi	Multi
1PL8	8	8	7305^{b}	8641^{bc}	747^{ab}	826^{bc}	7403^{b}	8295^{b}	2.71	1.71	101.0	94.3
1BST	8	8	8253^{a}	10356^{a}	813^{a}	1001^{a}	8254^{a}	10096^{a}	2.0	1.75	96.4	94.8
3PBL	8	8	6617^{b}	7453^{c}	649^{c}	735^{c}	6740^{b}	7563^{c}	3.38	2.63	116.5	99.1
3BST	8	8	7011^{b}	8818^{b}	721^{ab}	883^{b}	7306^{b}	9111^{b}	1.67	3.17	78.8	119.5

^{abc}Means in same column differ (p<.05); [1]Cheese Yield; [2]3.5% fat correct milk; [3]Days open

Table 2

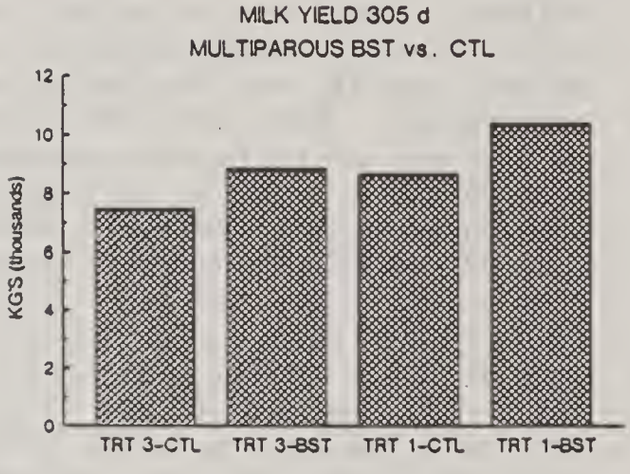


Figure 1

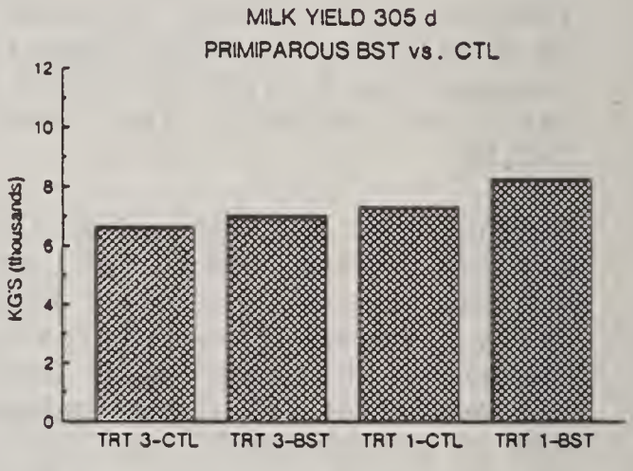


Figure 2

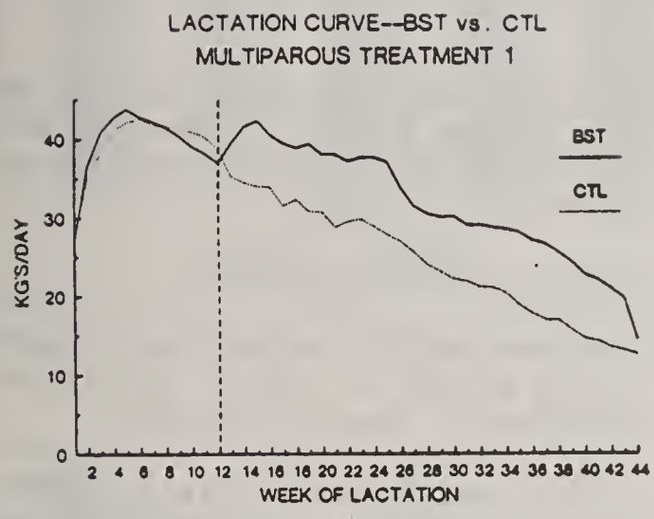


Figure 3

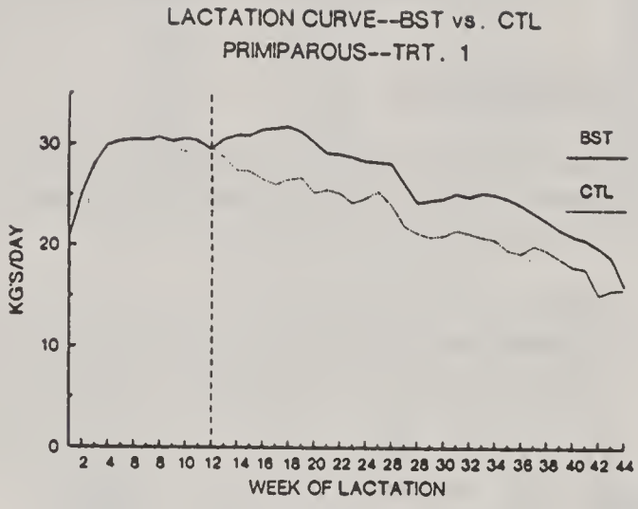


Figure 4

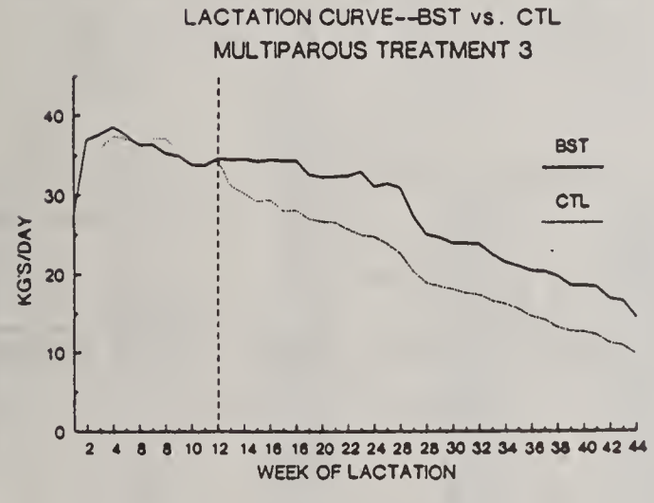


Figure 5

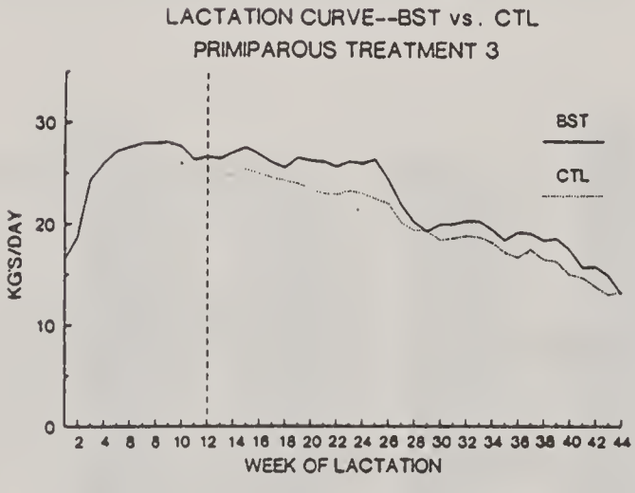


Figure 6

EFFECT OF DIETARY FORAGE:GRAIN RATIO AND BOVINE SOMATOTROPIN ON DRY MATTER DIGESTIBILITY AND RETENTION TIME OF FORAGE IN THE RUMEN

J. KLEINMANS, T.R. DHIMAN, H.D. RADLOFF, N.J. TESSMANN AND L.D. SATTER

INTRODUCTION

The most important source of variation in NE value of feeds is caused by variation in digestibility.

The objective of this study was to determine digestibility of diets containing different forage to grain ratios in early and late lactation and with or without BST in mid lactation.

MATERIALS AND METHODS

Ration dry matter digestibility (DMD) and rate of passage (ROP) of alfalfa silage (AS) were estimated with cows and heifers in early, mid and late lactation using Yb and La as indigestible markers. In early lactation ROP and DMD were estimated in five diets with different forage to grain ratios, and in late lactation four diets were used. In mid lactation these measurements were estimated in two diets, each divided between placebo (injection of saline solution) and BST treatment (injection of 20.6 mg rBST). See Neal J. Tessmann et al. p. 46 and 49 for further details of experimental design.

RESULTS AND DISCUSSION

Increasing the proportion of alfalfa silage in early lactation resulted in lower ration DMD.

In mid lactation the effect of BST on digestibility was tested in two different diets. There was no effect of BST treatment on DMD (Fig. 2).

In late lactation there were no differences in DMD between diets with 68% or 98% alfalfa silage for cows. Heifers, however, reacted differently. The increasing amount of forage caused a linear decrease in DMD. (Fig. 3).

Retention time of Lanthanum (applied to alfalfa silage) in the rumen was not affected by the amount of alfalfa in the diet or by administration of BST (Fig. 4-6).

Table 1. Number of animals in early, mid and late lactation.

	weeks in lactation								
	7-12			19-25			30-40		
Treatment	% AS*	Cows	Heifers	% AS*	Cows	Heifers	% AS*	Cows	Heifers
1 Placebo	38.2	8	16	48.2	6	8	68.2	5	6
1 BST	--	--	--	48.2	7	8	--	3	4
2	48.2	7	8	--	--	--	78.2	7	7
3 Placebo	58.2	11	15	68.2	8	8	88.2	5	6
3 BST	--	--	--	68.2	7	8	--	3	4
4	68.2	8	8	--	--	--	98.2	6	7
5	98.2	9	6	--	--	--	98.2	6	7

* alfalfa silage in total mixed ration.

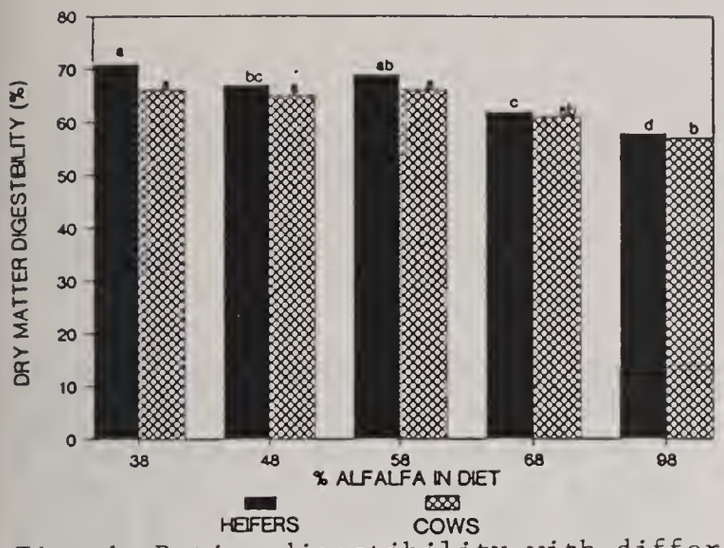


Fig. 1. Ration digestibility with different forage to grain ratios in early lactation.

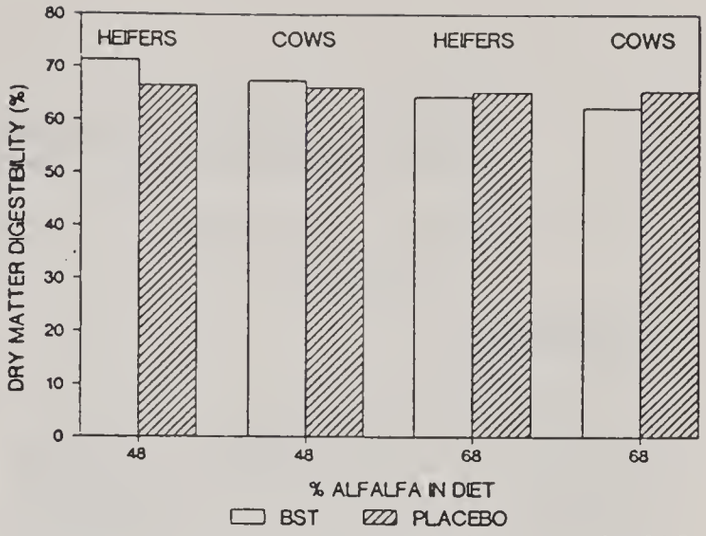


Fig. 2. Ration digestibility with different forage to grain ratio with or without BST in mid lactation.

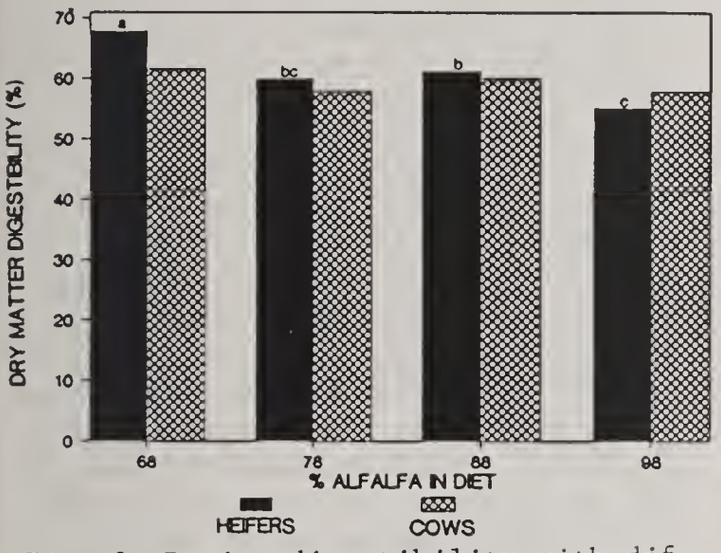


Fig. 3. Ration digestibility with different forage to grain ratios in late lactation.

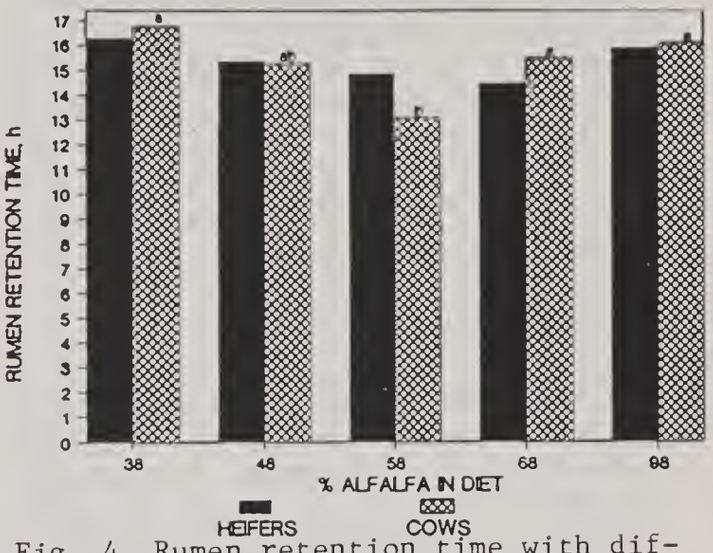


Fig. 4. Rumen retention time with different forage to grain ratios in early lactation.

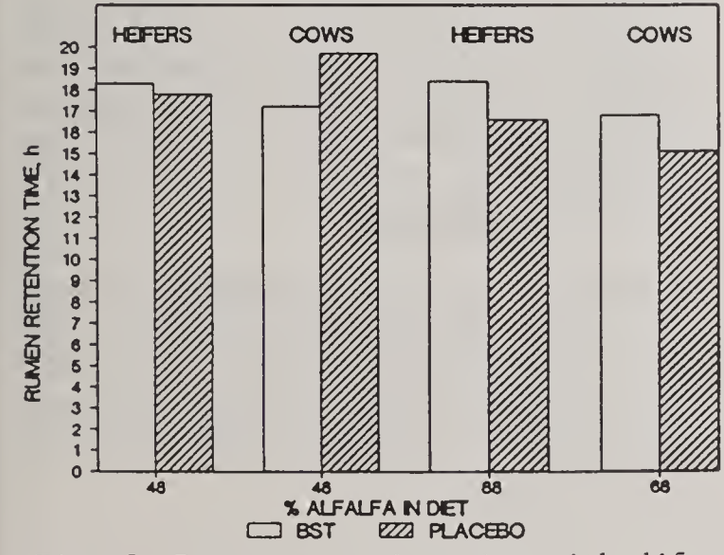


Fig. 5. Rumen retention time with different forage to grain ratios with or without BST in mid lactation.

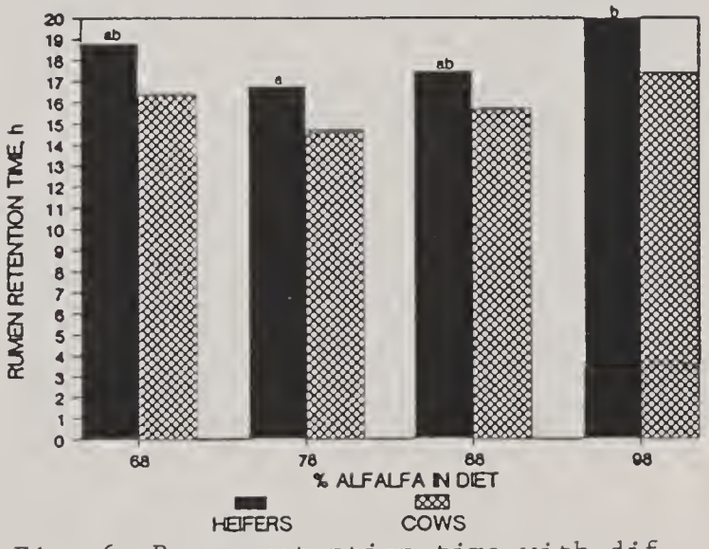


Fig. 6. Rumen retention time with different forage to grain ratios in late lactation.

EFFECT OF DIETARY FORAGE:GRAIN RATIOS ON BLOOD METABOLITES IN HIGH PRODUCING DAIRY COWS FED HIGH QUALITY ALFALFA SILAGE

T.R. DHIMAN, J. KLEINMANS, N.J. TESSMANN, H.D. RADLOFF AND L.D. SATTER

INTRODUCTION

Marked alterations in nutrient metabolism occur at the start of lactation in order to accommodate demands of the mammary gland. If the animal is unable to alter nutrient metabolism rapidly enough or to the extent needed to meet demands for milk synthesis, either the cow will produce well below her capabilities or she will develop various metabolic disorders and health problems. The objective of this study was to measure the effect of different forage:grain ratios on blood metabolites.

MATERIALS AND METHOS

The number of animals and percentage of forage (dry basis) in total mixed diets at different stages of lactation is given in Table 1 and 2 on page 47. Both primiparous and multiparous cows were assigned to the treatments. Blood samples were collected at week 2,3,4,6,8,12 and 16 of lactation and were analyzed for glucose, urea, β - hydroxybutyrate and plasma free fatty acids.

RESULTS AND DISCUSSION

Average blood glucose concentrations in primiparous and multiparous cows with different forage:grain ratios in treatments 1 through 5 are shown in Figures 1 and 2. Blood glucose concentration was significantly lower ($P<0.05$) with the all forage diet (98.2% alfalfa silage) than the low forage diet (38.2% alfalfa silage) until 8 weeks of lactation in multiparous cows and until 12 weeks in primiparous cows. Different dietary forage:grain ratios did not alter the blood urea-N.

Plasma β-hydroxybutyrate (BHB) concentration was affected by different forage:grain ratios in both multiparous and primiparous cows. Increasing the proportion of forage in the diet resulted in increased concentration of BHB. As the lactation advanced the difference between treatments was narrowed and by week 12 and 16 of lactation the difference between treatments was diminished (Figures 3 and 4). High concentrations of BHB in plasma in early lactation indicates that a large amount of energy was being utilized to meet the energy demands in early lactation.

Dietary forage:grain ratio did not affect plasma free fatty acid (PFFA) concentrations. However, in early lactation there were higher concentrations of PFFA in all the treatments and it declined as lactation advanced.

Feeding cows with high forage diets in early lactation resulted in reduced energy intake, and cows were dependent on body energy reserves to meet their energy demands.

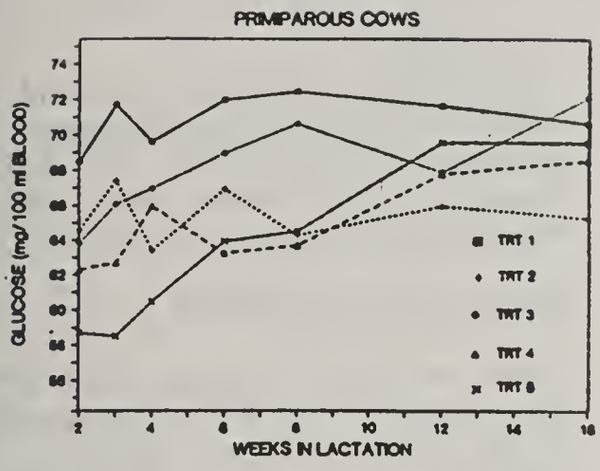


Figure 1

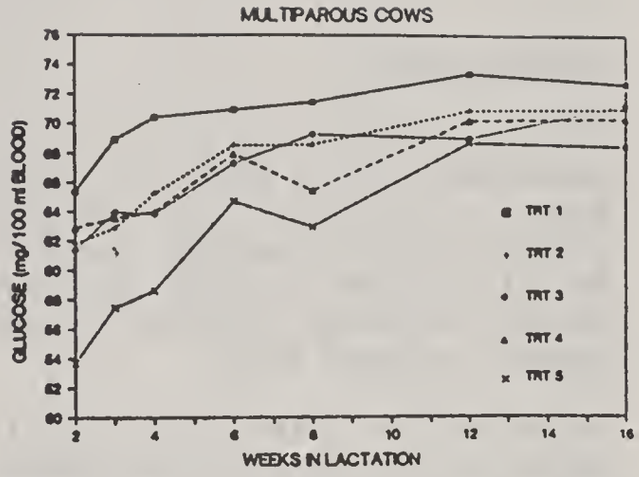


Figure 2

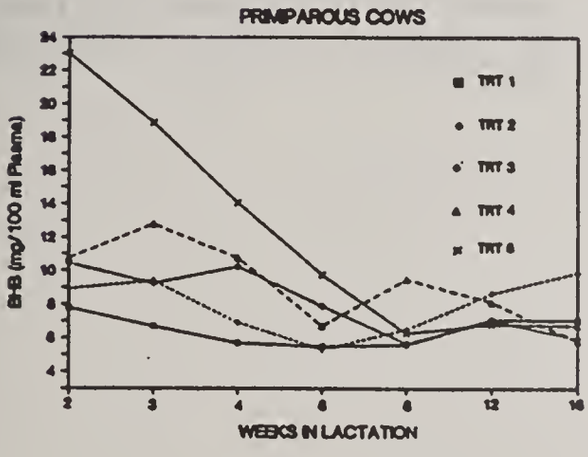


Figure 3

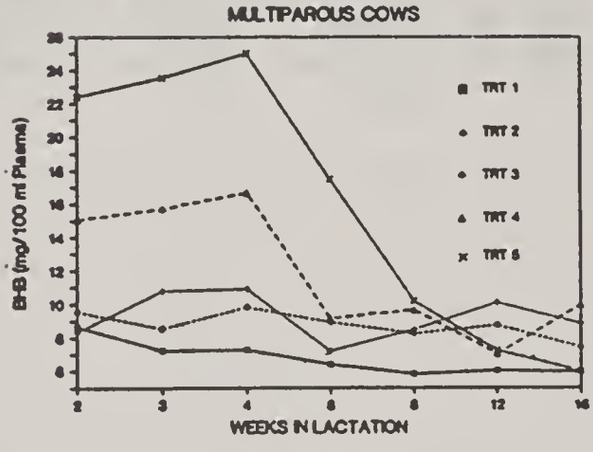


Figure 4

EFFECT OF DIETARY FORAGE:GRAIN RATIO ON DIGESTION MEASUREMENTS AND DIGESTA PASSAGE RATE IN LACTATING DAIRY COWS

J. KLEINMANS, T.R. DHIMAN, H.D. RADLOFF AND L.D. SATTER

INTRODUCTION

Little information on digestion parameters exists with all forage diets containing high quality alfalfa, or on digestion kinetics of diets containing variable amounts of grain, especially with respect to rumen fill.

The objective of this study was to investigate digestion parameters and rumen fill of an all forage diet vs. diets with various amounts of grain supplementation.

MATERIALS AND METHODS

Four lactating cows fitted with rumen cannulas were fed total mixed rations containing 38%, 58%, 78% or 98% high quality forage in a 4X4 Latin square design. Ruminal digestion kinetics were determined by the in situ dacron bag method. Rate of passage of alfalfa silage, high moisture corn and liquid were estimated using La, Sm and Cr-EDTA as indigestible markers. Yb was used to determine dry matter digestibility. Rumens were emptied manually for rumen fill data.

RESULTS AND DISCUSSION

Rumen retention time (RRT) of La and Sm were not affected by different forage to grain ratios. Cr-EDTA appeared to have a lower RRT with increasing proportion of alfalfa silage, however, this difference was not significant.

The rate of NDF digestion was significantly lower with high grain diets, indicating better conditions for microbial breakdown of NDF with high alfalfa diets.

Dry matter intake was lower and rumen fill was higher with all forage diets, however, differences in rumen dry matter content were small.

Measurement	Percent Alfalfa in Diet			
	38	58	78	98
Diet Chemical Analysis, CP%	20.5	21.2	21.9	21.1
NDF%	27.4	32.4	37.5	42.7
ADF%	18.4	23.9	29.3	34.7
Dry Matter Intake, kg/d	23.0^{a}	23.6^{a}	22.1^{ab}	20.2^{b}
% BW	3.73^{a}	3.77^{a}	3.67^{a}	3.22^{b}
Dry Matter Digestibility %	63.4^{ab}	65.0^{a}	53.4^{c}	55.0^{bc}
Milk yield, kg/d	37.7^{a}	35.8^{a}	32.8^{b}	27.2^{c}
Milk Fat, %	3.57	3.16	3.38	3.47
Milk Protein, %	3.00^{a}	2.79^{cb}	2.83^{b}	2.70^{c}
Rumen Fill:				
Wet Digesta, kg	67.4^{a}	72.7^{b}	74.5^{b}	86.3^{c}
Dry Digesta, kg	10.2^{a}	10.4^{ab}	10.0^{ab}	11.6^{b}
Ruminal Retention Time ($1/k_p$):				
La (forage), h	11.5	10.2	10.0	11.1
Sm (grain), h	12.0	12.2	10.6	--
Cr-EDTA (liquid), h	5.4	4.7	4.2	4.0
In Situ Digestion				
Alfalfa NDF:				
Potentially digested, %	49.8	50.1	49.1	49.0
Rate (k_d), h^{-1}	.054^{a}	.066^{ab}	.089^{bc}	.106^{c}
High Moisture Ear Corn DM:				
Solubilized or removed from bag at 0 hr, %	66.0	65.4	63.7	66.3
Potentially digested insoluble DM, %	26.0	28.2	30.2	28.4
Rate (k_d), h^{-1}	.067	.071	.069	.071

EFFECT OF DIETARY NITROGEN SOURCE ON CONCENTRATIONS OF FLUORESCAMINE-REACTIVE PEPTIDES IN THE SHEEP RUMEN

G.A. BRODERICK AND R.J. WALLACE

INTRODUCTION

Small molecular weight nitrogen (N) compounds found in the rumen originate from the diet and during degradation of dietary protein. Ruminal concentrations of the protein metabolites ammonia and amino acids have been studied extensively. The most heterogeneous, and least understood, intermediates of ruminal protein catabolism are peptides. Peptides are required for growth of the rumen anaerobe Bacteroides ruminicola, and peptides give rise to more rapid growth than ammonia alone with mixed rumen organisms. Mixed rumen organisms have been reported to degrade peptide-bound amino acids more rapidly than free amino acids (FAA). A fluorimetric method was developed for determination of peptides in ruminal fluid and this procedure was used to study the influence of different N sources on ruminal peptide levels.

MATERIALS AND METHODS

Four sheep, weighing 50-55 kg and fitted with permanent ruminal cannulae, were fed twice daily (at 0800 and 1600 h) .5 kg of a diet consisting of 67% hay and 33% concentrate. The concentrate contained supplemental N from urea, casein or ovalbumin, which provided 32, 34 or 34% of the total crude protein in the respective diets. The trial consisted of three, 4-wk periods. All sheep were fed the urea diet for the first 4 wk, then two were switched to the casein diet and two to the albumin diet for the next 4 wk. The protein-containing diets were switched during the last 4 wk of the trial. Ruminal contents were sampled via cannulae just prior to feeding (0 h), and at 1, 2, 3, 4, 6 and 8 h after feeding; contents were filtered through 4 layers of muslin. The strained ruminal fluid (SRF) was treated with trichloroacetic acid, and analyzed for ammonia and total FAA using conventional methods.

Peptide concentrations were determined in SRF by reaction with fluorescamine at pH 6.2. Fluorescence of individual amino acids in this procedure was typically only 1 to 2 % that of corresponding peptides. Glycine, the free amino acid showing the greatest response, yielded 8 and 5% of the fluorescence of di- and triglycine, respectively. Although fluorescence differed for specific peptides (Table 1), response was linear for each peptide studied. Trialanine response was typical for the tri- and oligopeptides tested; therefore, it was selected as standard and SRF peptides are reported as trialanine equivalents. Mean recovery of trialanine added to SRF was 93.4 (SE 3.0)%, and peptide concentrations in SRF were corrected for trialanine recovery. Mean (SE) peptide concentration in Bactocasitone, a source of mixed peptides from partial hydrolysis of casein, was determined to be 13.6 (SE .66) μmol trialanine equivalents/mg N. The average peptide size was computed to 4.0 amino acids/peptide.

RESULTS AND DISCUSSION

The effects of diet on ruminal concentrations of ammonia were not unexpected. Ammonia rose to very high levels by 1h after feeding the urea diet, before declining rapidly in a first-order manner; levels were not different between urea-fed and ovalbumin-fed sheep by 4h. Peak ammonia levels were next highest on casein (maximum at 2h), and lowest on ovalbumin (maximum at 3-4h). Levels were the same on all three diets at 0-h, and all had returned to prefeeding levels by 6h. The higher ammonia concentrations with casein than ovalbumin reflect the known greater resistance of ovalbumin to ruminal degradation.

Concentrations of FAA were much lower than ammonia; the maximal level (with casein) was 1.4mM versus maximal ammonia (with urea) of 38mM. Concentrations of FAA peaked rapidly after feeding casein, before declining rapidly. However, it is surprising that FAA levels were next greatest with urea than with ovalbumin. Ovalbumin feeding did not result in significant change in FAA from prefeeding levels.

Ruminal concentrations of peptides, determined using the fluorescamine method, were much greater on the casein diet. Peptides were low (.2-.3 mM) and essentially constant on the urea and ovalbumin diets (Table 2). However, peptides increased dramatically to 3.8mM at 1h after feeding casein before declining to prefeeding levels by 3h. This rapid in vivo clearance of casein peptides is interesting. Some workers reported that peptides from in vitro casein degradation were still elevated after 7h of incubation; however, others found that peptides did not accumulate during ruminal degradation of soluble protein from alfalfa leaves.

In this experiment peptides accumulated with feeding of rapidly degraded casein, but not with more slowly degraded ovalbumin. These results also suggest that peptide metabolism may influence overall rate of protein catabolism in the rumen.

TABLE 1. RELATIVE FLUORESCENCE OF SPECIFIC PEPTIDES IN FLUORESCAMINE ASSAY

Peptide[a]	Relative Response[b] $\bar{X}$	SE
Dipeptides		
GlyAla	6.7	.3
GlyGly	7.7	.2
GlyPro	8.1	.3
AlaAla	9.4	.2
ValAla	17.7	.7
Tri- and Oligopeptides		
AlaGlyGly	3.7	.5
GlyGlyAla	8.2	.9
GlyGlyLeu	9.2	.4
GlyAlaAla	10.0	.1
GlyGlyPhe	11.2	.9
AlaAlaAla	12.1	.4
AlaAlaAlaAla	12.1	.2
AlaAlaAlaAlaAla	12.4	.3
LeuGlyGly	14.3	.6
PheGlyGly	27.1	1.5

[a]All optically active amino acid residues in peptides were of the L-configuration.
[b]Fluorimetric response (FR) of peptide relative to that of quinine sulfate. Relative response = [(FR/μmol peptide)/(FR/μg quinine sulfate)].

Table 2. Ruminal peptide concentrations after feeding different supplemental-N sources.

Time	Supplemental-N source		
	Urea	Casein	Ovalbumin
(h)		(mM)	
0	.21[b]	.19[a]	.20[b]
1	.29[b]	3.80[a]	.31[b]
3	.23	.22	.24
8	.22	.17	.21

[a,b]Means at 1 h with different superscripts are different (P<.05).

ISOLATION OF MONENSIN-SENSITIVE RUMEN BACTERIA WITH HIGH CAPACITIES OF AMMONIA PRODUCTION

J.B. RUSSELL, H.J. STROBEL AND G. CHEN

INTRODUCTION

Amino acid deamination by rumen microorganisms is a nutritionally wasteful process that often yields more ammonia than can be used in microbial growth. The use of insoluble feed products can decrease ammonia production, but many feed ingredients (e.g. forages and soybean meal) contain an abundance of soluble proteins which ferment rapidly in the rumen. Ionophores that inhibit Gram-positive bacteria and protozoa also decrease deamination, but the mechanism of this action had never been satisfactorily explained. Protozoa are also sensitive to monensin, but recent work indicated that their capacity of ammonia production is less than the bacteria.

In the early 1960's, Bladen et. al. (1961) examined the capacity of predominant rumen bacteria to produce ammonia. Specific activities were not reported, but the amount of ammonia produced was not great even though the incubation period was 96 h. Because the mixed cultures had activities that were significantly higher than previously isolated pure cultures, we undertook experiments to enrich and isolate monensin-sensitive rumen bacteria with high specific activities of ammonia production.

MATERIALS AND METHODS

Mixed rumen microorganisms were obtained from a 600 kg nonlactating dairy cow that was fed 2.5 kg timothy hay and 2.5 kg commercial concentrate supplement containing 16% crude protein twice daily. Bacterial enrichments initially contained lactate, dulcitol, pectin, or xylose (1 g per liter) and Trypticase (1 g per liter). Later enrichments contained 15 g per liter Trypticase as the sole energy and nitrogen source. Once ammonia production of the enrichments had stabilized (usually 6 to 10 days), they were grown on agar plates containing basal medium and the same energy source. After 1-3 days, isolated colonies of different morphology were picked.

Standard tests were used to identify the isolates. Volatile fatty acids, lactate, succinate, and sugars were determined by high pressure liquid chromatography. Hydrogen gas was measured with a thermal conductivity gas chromatograph. Ammonia was measured by the colorimetric method of Chaney and Marbach (1962). Chromosomal DNA was isolated using a modification of the method of Berns and Thomas (1965). The midpoint melting temperature was used to determine the guanine plus cytosine (G+C) content of the DNA.

RESULTS

When mixed rumen bacteria were inoculated into semi-continuous cultures (25% transfer every other day) containing lactate, dulcitol, pectin or xylose and Trypticase (1 g per liter) as the sole nitrogen source, the specific activity of ammonia production increased. The greatest enrichment was observed with lactate and xylose and in these cases the specific rate of

ammonia production was 8 fold higher than the rumen fluid control (approximately 35 nmol ammonia per mg protein per min). Isolates of different morphology were obtained from each of the enrichments, but in no case did the specific activity of the isolate exceed mixed rumen bacteria. If Trypticase (15 g per liter) was used as the only energy and nitrogen source, there was an even greater increase in ammonia production, and 2 bacteria, a peptostreptococcus and a clostridium were obtained. The peptostreptococcus was unable to grow on any of 25 carbohydrate or carbohydrate derivatives tested, but the clostridium was able to use glucose, maltose, fructose, cellobiose, trehalose, sorbitol and salicin as energy sources. Neither organism was able to grow in the absence of an amino acid source, but growth rates on Trypticase were >0.35 h^{-1}. The specific activities of ammonia production were 346 and 427 nmol per mg protein per min for the peptostreptoccoccus and clostridium, respectively. Megasphaert elsdenii and Bacteroides ruminicola, previously isolated ruminal ammonia producers, had specific activities of only 11 and 19 nmol ammonia per mg protein per min, respectively. The most probable number of the colstridium in rumen fluid was $<10^3$ per ml, but the peptostreptococcus was present at 10^8 per ml. Since the peptostreptococcus was present at significant numbers and was a very active ammonia producer, it could play an important role in the wasteful fermentation of dietary amino acids.

DISCUSSION

The ionophore, monensin, appears to decrease ammonia production and spare dietary protein from fermentation, but it had little effect on the best previously isolated ammonia-producing bacteria. The isolation of a predominant, Gram-positive, monensin-sensitive bacterium with a very high specific activity of ammonia production appears to clarify the contradiction between the rate of ammonia production in vivo and the specific activities of pure cultures. Monensin is used extensively in the beef cattle industry, but the antibiotic has not been approved for lactating dairy cattle. Work is currently being conducted to see if this ammonia producing organism can be inhibited by methods other than antibiotic treatment.

DEVELOPING A STANDARD PROTOCOL FOR IN VIVO FORAGE EVALUATION

D.R. MERTENS AND D.J. CHERNEY

INTRODUCTION

Routine evaluation of forages using dairy cows is limited due to the large quantity of forage required per cow and the expense of dairy cow trials. Dairy cow rations also typically contain concentrates making measurement of forage evaluation difficult. Sheep are commonly used for digestibility trials, but the value of the results for evaluating forages for dairy cows is often questioned. The digestive physiology of the two species is similar, but feed intake is typically less for sheep than dairy cattle. High feed intake has

been associated with digestibility depression in dairy cows, indicating a need for forage evaluation methods that relate intake and digestibility. For these reasons, developing standard protocol using sheep that can be used to estimate intake and digestibility of forages by dairy cows is desirable. This report describes a sheep trial protocol that varies intake in order to estimate forage quality for dairy cows.

MATERIALS AND METHODS

Growing wether sheep ranging in age from 6-18 months are utilized in all trials to insure that daily ad libitum intakes of high quality hay range from 2 to 4% of BW. Sheep are penned inside 10 days prior to the start of each trial during which time they are fed high quality alfalfa hay. One week prior to the initiation of each trial, sheep are wormed, sheared, and assigned to a test forage based on body weight. Since animals are sometimes used for more than one trial, care is taken that no sheep is assigned to the same forage or metabolism crate within an experiment. No forage is assigned to the same crate location in the barn across periods. Sheep are fed once a day, have free access to water and TM salt, and have freedom of movement in the crates.

Periods consist of 42 days. All sheep are fed a reference standard hay (high quality alfalfa) during days 1-10 (L0). Intake and digestibility of this reference hay is used as a covariate to adjust for animal differences within and among experiments. Sheep then are fed a test forage at three levels: (1) ad libitum, allowing a 10 to 20% refusal (L1, days 11-24), (2) 100% of what was eaten during ad libitum level (L2, days 25-31) and (3) offered hay at 1.8% of body weight (L3, days 32-40). Hay intakes at 1.8% of body weight per day corresponds to maintenance levels of feed intake for sheep of the size and age used in these trials.

Forage dry matters (105°C) are determined weekly during each trial. Forage offered is sampled daily (300g), composited and dried at 55°C during collection phases. Hay refused is weighed daily (all barn weights to the nearest gram), composited and frozen until it can be dried at 55°C for each collection phase. Waste forage is collected on screens under each crate, recovered at the end of each collection phase, weighed and frozen until dried at 55°C. Feces are collected twice daily in canvas bags harnessed to the sheep. A 20% aliquot is collected and pooled during the digestibility phase of each level. Feces are frozen and stored until dried at 55°C. Subsamples of feed offered and refused and feces are dried to 105°C for determination of dry matter intake and digestibility.

RESULTS AND DISCUSSION

New laboratory methods of forage evaluation must be validated with in vivo results. Since laboratory analysis can only measure the characteristics of the feed and not influences of the animal to which it is fed, it is critical that the laboratory methods be compared to in vivo digestibility that is measured under conditions that minimize animal effects. Thus, digestibility measured when feed is offered at a constant 1.8% of body weight per day (L3) will be related to laboratory measures because it reduces variability due to selection and intake differences

among animals. It also relates most closely what is eaten to what is sampled and analyzed in the laboratory. Ad libitum intake is not a constant that is characteristic of a feed; rather it varies with the physiological status of the animal to which it is fed and the degree of selection and refusal the animal is allowed. Thus, the standard protocol assesses the physiological status of each animal using the reference hay (L0) and fixes the level of refusal or overfeeding to a constant (L1). Using forage intakes measured in this way will allow more accurate determination of the feed characteristics, measured in the laboratory, that are related to intake.

Forage evaluation for use in formulating dairy rations must be measured at ad libitum levels of intake. In order for sheep to be adequate models for dairy cow forage evaluation, the protocol must determine the relationship between sheep intake and digestibility and the relationship must be similar to that observed in dairy cows. The restricted ad libitum period (L2) provides a third measure of digestibility and intake with more limited selection of the diet than measured in L1 to minimize that source of animal variation. If the relationship between intakes, selection and digestibility is similar between cattle and sheep, then sheep data can be used to predict the digestibility of forages by dairy cows at any specified level of intake.

The protocol has been used in an experiment in which 12 hays with similar NDF compositions (4 barley, 4 oat, 2 pearl millet and 2 sorghum X sudan were used. Dry matter digestibility, intake and % refusal averaged across treatments is reported in table 1. Digestibility decreased with increased intake suggesting that sheep may respond similarly to lactating cows, and the protocol for in vivo forage evaluation can be used to determine the digestibility of forages by high producing dairy cows. Research is in progress to compare sheep and cattle estimates of digestibility of forages using the standard protocol for in vivo forage evaluation.

Table 1. Average dry matter digestibility (DMD), intake (DMI) and % refused at three levels of intake.

LEVEL	DMD	DMI	% REFUSED
L1	62.3	2.12	17
L2	63.4	1.92	11
L3	64.2	1.66	5

MODIFICATIONS OF THE DETERGENT FIBER SYSTEM FOR MEASURING SULFURIC ACID LIGNIN

R.S. CARDOZA and D.R. MERTENS

INTRODUCTION

Current lignin analyses are method dependent and vary in purity. Core lignin, the insoluble polymer of phenylpropane units, is often contaminated with cutin, protein, Maillard products, tannins and ester-linked phenolic acids that condense during extraction procedures. Although these latter two compounds are phenolics, they may not be associated with the core lignin matrix and may have different relationships with forage cell wall digestibility.

In general, procedures giving minimal yields of lignin are preferred because it is assumed that these methods minimize contamination. Differences between published lignin estimates from various lignin methods may be due to contamination by protein and/or labile phenolics. Detergent extraction in the regular and sequential detergent fiber systems reduces but may not eliminate contamination of core lignin. The objective was to determine the effects of protease incubation and alkali extraction on the recovery of 72% H_2SO_4 lignin.

MATERIALS AND METHODS

The experiment utilized a 2x3x4x6 factorial arrangement of treatments in a randomized complete block design. Samples were extracted sequentially using neutral detergent (ND) followed by acid detergent (AD) prior to 72% H_2SO_4 lignin recovery. The two drying temperatures between extractions were room temperature (23°C) 105°C in a forced-draft oven. Three protease treatments consisted of no protease, protease prior to ND and protease following ND. Four alkali treatments included no alkali, alkali prior to ND, alkali between ND and AD and alkali following AD. Six sample sources were a high-tannin legume (birdsfoot trefoil), a low-tannin legume (alfalfa), a cool-season grass (barley), a warm-season grass (pearl millet), and feces from sheep fed the corresponding alfalfa and barley hays. All samples were dried at 50°C and ground through a 1mm Wiley mill.

To aid in the removal of nitrogenous compounds, a type XIV bacterial protease (Pronase E) from Streptomyces grieseus (Sigma P-5147) was incubated with the samples in 0.05M Trizma (pH 7.4). Samples were rehydrated by incubating in 40 ml of buffer for 30 minutes at 37°C. After rehydration, a solution of 5 mg protease in 10 ml of buffer was added and allowed to incubate at 37°C for 60 minutes. Residues were filtered into coarse-porosity, fritted-disk Gooch crucibles and rinsed with hot water.

Alkali-labile phenolics were extracted using a 1M NaOH solution. Samples were extracted for 24 hours in 20 ml of alkali at room temperature. Residues were filtered into fritted-disk crucibles and rinsed with hot water.

Crucibles receiving fecal residues contained Whatman GF/D filter mats (4.5 cm) to aid in filtering.

Blank crucibles, both with and without mats, were used to correct for day-to-day variation in weighing. All lignin and ash residues were measured using a hot-weighing technique (dried at 105°C for a minimum of 12 hours) with a computerized weigh acquisition system. One gram forage and 0.5 gram feces samples were randomly assigned to the crucibles for each protease X alkali combination. To minimize variation between extractions, all reagents were prepared prior to the onset of the experiment and all analyses were performed by one person. Each factorial combination of treatments was replicated twice. Replications were blocked to detect extraction differences due to time of analysis.

RESULTS

Regular (8.0%) and sequential (7.6%) detergent extraction resulted in higher recoveries of 72% H_2SO_4 lignin compared to either protease (7.4%) or alkali (6.4%) treatments alone. The combination of protease and alkali additions prior to ND extraction (5.7%) resulted in lower lignin recoveries than when applied after ND (6.3%). Protease had no effect after ND while the reduction in lignin associated with alkali extraction decreased in order of treatment: NAL>NAKL>NKAL>KNAL. Recovery of lignin is lowest with a combination of protease and alkali treatments prior to ND extraction. Reduction of lignin was 24.6% of the sequential lignin estimate.

Protease applied prior to ND extraction lowered 72% H_2SO_4 lignin recoveries for alfalfa and pearl millet hays and both feces, whereas barley and birdsfoot trefoil were unaffected by protease treatment sequence. Alkali lowered the recovery of lignin for all samples but the magnitude is influenced by sequence location. Alkali extraction after AD had little effect on feces but lowered lignin recovery for both birdsfoot trefoil and pearl millet.

Drying intermediate residues at 105°C resulted in higher 72% H_2SO_4 lignin than drying at 23°C but this amounted to only 0.6 mg/g (1% difference). The lower drying temperature resulted in lower recoveries of lignin with alkali whereas protease was unaffected.

CONCLUSIONS

Protease prior to ND extraction may remove protein from feces and alfalfa that is resistant to detergent solubilization making it an alternative to the use of sodium sulfite in the ND procedure. Alkali extraction decreases lignin recoveries, especially if used before AD. This effect is greater for grasses than legumes, suggesting that alkali-labile phenolics in grasses were removed. Similarily, the lignin content of birdsfoot trefoil was lowered by alkali indicating that tannins may not be completely removed by either ND or AD. The traditional ADL appears to overestimate core lignin. Sequential extraction with ND prior to AD lowers lignin values and combining this technique with protease and alkali results in lower lignin concentrations which may more accurately measure the core lignin fraction.

Effects of extraction methods on neutral and acid detergent fiber and 72% sulfuric acid lignin[1].

Treatment Sequence	%NDF	%ADF	%SLIG
A-L	--	40.3	8.0
N-A-L	62.2	38.9	7.6
N-AK-L	63.2	34.5	6.9
PN-A-L	60.1	38.3	7.4
NP-A-L	61.0	38.4	7.4
PN-KA-L	62.5	34.4	6.2
NP-KA-L	60.0	34.0	6.3
PN-AK-L	62.1	34.4	6.7
NP-AK-L	61.0	34.0	7.0
KN-A-L	41.4	35.1	6.1
NK-A-L	40.8	34.6	6.4
KNP-A-L	41.6	35.0	6.3
PKN-A-L	37.2	31.8	5.7

[1] = weighing point in the sequence
A = acid detergent extraction
N = neutral detergent extraction
P = pronase incubation
K = alkali extraction

REDUCTION OF ERROR IN NIRS ANALYSIS VIA CLUSTER SUBSETTING

S.M. ABRAMS

INTRODUCTION

A previous attempt was made to utilize cluster analysis as a means for reducing the number of samples needed for NIRS calibration. By allowing statistical techniques to analyze the structure of the data set, and then proportionally sampling from the components of that structure, it was thought that a better representation of the population of samples available for calibration could be achieved. Under the conditions of that experiment, no improvement was seen by selecting samples from each cluster, vs. random selection. Another potential use of cluster analysis would be to improve calibrations by establishing separate calibration equations for each cluster, determining which cluster each unknown belongs to (discriminant analysis), and using the appropriate equation to determine the composition of the unknown. This technique was tested on a structured data set in this experiment.

MATERIALS AND METHODS

Some 567 samples were obtained from a factorially arranged field study. There were five species (alfalfa, red clover, birdsfoot trefoil, orchardgrass and timothy), two years and three cuttings. Samples were subjected to oven drying at

three temperatures or freeze drying after various periods of field drying. One hundred samples were taken at random to be the validation set. Cluster analysis was utilized on the remaining samples, using FASTCLUS of SAS. The number of clusters were allowed to equal 1 (no clustering), 2, 4, 6 and 8, using spectral reflectance as the clustering variables. Stepwise regression (6 wavelengths) was used to create calibration equations for each cluster, within each cluster analysis. The mean weighted standard error of calibration (SEC) for each chemical analysis was calculated within each cluster analysis. The minimum number of clusters to provide separate equations was then determined from where SEC seemed to plateau to a constant. Using calibration set spectra, labeled with cluster number, and unknown spectra, discriminant analysis was used to place unknowns into appropriate clusters. Finally, appropriate equations (those associated with the particular cluster) for each unknown were used to measure the chemical composition of the unknown.

RESULTS AND DISCUSSION

Table 1 shows the response of mean SEC to clustering. SEC's appeared to plateau at 6 for protein and IVDMD, at 2 for ADF and at 4 for NDF. It was decided to utilize 6 clusters for each constituent, forming calibration equations for each cluster. Table 2 shows the results when all 464 samples were used to generate calibration equations versus the results from cluster equations. This technique reduced SEV by 25, 30, 24 and 15% for protein, ADF, NDF and IVDMD, respectively. Bias for NDF was reduced by 55%. This data set was a highly structured set, in which clustering may have a particular advantage. However, such sets are common in experimental studies and the technique may have particular benefit in large experimental studies which permit the grouping of samples into subsets. Further refinement of this technique will be addressed in the coming year.

Table 1. Mean weighted standard error of calibration for establishment of calibration equations on cluster subsets.

Number of clusters	CP	ADF	NDF	IVDMD
1	.74	1.88	2.32	1.96
2	.67	1.30	1.92	1.84
4	.58	1.13	1.63	1.60
6	.51	1.07	1.54	1.44
8	.49	1.00	1.52	1.39

Table 2. Validation statistics[a] determined from equations established with and without use of cluster analysis.

	RSQ		SEV		BIAS		SEVC	
Cluster:	No	Yes	No	Yes	No	Yes	No	Yes
CP	.97	.98	.78	.59	.04	-.03	.78	.59
ADF	.90	.95	1.75	1.23	-.04	.04	1.76	1.24
NDF	.97	.98	2.25	1.72	-.67	-.30	2.16	1.70
IVDMD	.89	.91	1.94	1.66	-.14	.11	1.94	1.67

[a]RSQ=coefficient of determination, r^2; SEV=standard error of validation; BIAS=difference between mean of values determined by NIRS versus values determined by conventional chemistry; SEVC=standard error of validation corrected for bias.

MICRO-ANALYSIS OF DERIVATIZED OXALIC ACID IN ALFALFA AND FESCUE BY REVERSE-PHASE HPLC

F.A. MARTZ, M. WEISS, M. KROHA and R.L. BELYEA

INTRODUCTION

The objective of this study was to develop a method to determine the oxalate content of forage samples weighing 250 mg. Several variations to the final method were used initially to develop the method. The method reported here is presented as we presently use it for total oxalic acid analysis in small forage samples.

MATERIALS AND METHODS

Plant Preparation: Plant materials were prepared by grinding individual air dried samples through a 20 mesh screen using a small stainless steel Wiley mill. Ground plant materials were stored in screwtop plastic containers at 24-26°C.

Extraction Procedure: A 250 mg sample of plant material was weighed to the nearest 0.1 mg. Plant samples were weighed into 50 ml erlenmeyer flasks that had been pre-tared to the nearest 0.1 mg and 30 ml of 1.0 N HCl was then placed into each flask, boiled for 18 minutes and allowed to cool to room temperature. Another option for extraction is the use of 30 ml 2X distilled water but not all types of oxalate will be extracted when only water is used.

After cooling the weight of the extract was recorded to 0.1 mg. The extract was then gravity filtered into pretared 50 ml plastic screw top bottles using Whatman 11 cm #541 filter paper and 143 mm plastic microfunnels. Centrifugation at approximately 400

XG was substituted for the filtration step for selected samples with no apparent effect.

The pH was then adjusted to 3.0 using approximately 4.5 mls of 5 N NaOH while monitoring with a Fisher Accumet model 220 pH meter equipped with a Markson pencil size Tefmark combination electrode. One N NaOH was used to "fine tune" the pH after a reading of near 2.0 was observed. The volume was then adjusted to 40 ml (40 g) by weight with the addition of 2X distilled water until the tare weight plus approximately 40 grams had been achieved. Flask gross weight was recorded to the nearest 0.1 mg.

Enzymatic Incubation: Two 4 ml aliquots of filtrate were taken using a 4 ml volumetric pipet. Each aliquot was placed into a 13 X 100 mm glass screwtop culture tube that had been pre-tared with the cap to the nearest 0.1 mg. The tubes were again weighed along with caps. Total weight was recorded to the nearest 0.1 mg.

One tube received 0.5 ml of dilute buffer blank using a 1000 microliter Eppendorf pipet. Dilute buffer blank was prepared by diluting 10 mls of 0.1 M sodium acetate, pH 4, to 100 ml with 2X distilled water. The other tube received 0.5 ml of dilute buffer containing 0.2 Enzyme Units (E.U.) of oxalate decarboxylase (Sigma Chemical Co.). This solution was prepared by diluting 1 ml of sodium acetate, pH 4, containing 4 E.U. oxalate decarboxylase to 10 mls with 2 X distilled water. Rehydrated enzyme was stored in concentrated solution (4 E.U./ml 0.1 M sodium acetate, pH 4) and was held at 5°C. Each tube then received 0.5 ml 2X distilled water. All tubes were capped tightly with teflon faced rubber lined plastic caps, mixed and incubated 16 hours at 37°C in a covered bath.

Spiking Procedure: To measure recovery, selected samples were spiked with sodium oxalate or calcium oxalate in solutions to equal 22.5 ppm $(COOH)_2$ equivalent in the 40 ml extract. This was accomplished by substituting the 0.5 ml 2X distilled water prior to 37 C incubations with 0.5 ml of the appropriate conc. of sodium oxalate in 2X distilled water or calcium oxalate in 1 N HCl.

Spiking with calcium oxalate powder was always done into the dry plant material before extractions. Here also the the amount added was 22.5 ppm $(COOH)_2$ equivalent in the 40 ml extract.

Derivatization: Following the 37°C incubation, all tubes received 1 ml of 0.1 M o-phenylenediamine (Sigma Chemical Company) in 4 N HCl. The tubes were capped tightly, mixed and weighed to the nearest 0.1 mg. All tubes were then incubated 6 hours at 110°C in a gravity flow oven.

After 6 hours the tubes were removed from the oven, allowed to cool to room temperature and weighed to the nearest 0.1 mg. All samples and standards were refrigerated until they could be fractionated.

Fractionation: All samples were fractionated using Baker 10 SPE 1 ml disposable C-18 columns. The columns were first preconditioned with 1 ml of methanol, aspirated, followed by 1 ml of 2X distilled water, and again aspirated. Using a 1 ml volumetric pipet, 1 ml of sample (well mixed at room temperature) was pipeted onto the column and aspirated. Five hundred microliters of 2X distilled water

was pipeted on the column and aspirated before a collection tube was placed beneath the column. Then 500 microliters containing 85% 0.1 M ammonium acetate (HPLC grade and 15% methanol (HPLC grade) was injected onto the column, aspirated and collected. This step was repeated twice more so that the fraction collected had a volume of 1.5 ml. Fractions were labeled, tightly capped and stored at 5°C.

HPLC: All fractionated samples were analyzed using reverse phase HPLC with UV detection. A 20 microliter sample loop was loaded with 80 microliters of fractionated sample (4X loop volume used to minimize contamination) and injected onto a .46 X 25 cm, 10 micron, C-8 column (Perkin Elmer Corporation), maintained at 28°C. The solvent was 2X distilled water that had been filtered through a 0.2 micron Nalgene filter. The flow rate was 2.2 ml/min at less than 1800 psi.

A variable wavelength detector (Perkin Elmer model LC 75) was set at 314 nm with attenuation at 0.16 AUF. Peak area was obtained from a Perkin Elmer model M-2 minigrator while elution profile was monitored with a Perkin Elmer model 023 strip chart recorder.

RESULTS AND DISCUSSION:

The reaction of oxalic acid with o-phenylenediamine resulted in the derivative 2-3 dihydroxyquinoxaline. This derivative, because of its strong U.V. absorbance, was easily detected even in cases of low amounts of oxalic acid (ca. 30 ppm mid-range) in 40 ml extracts. However, oxalic acid was not the only compound in plant extracts that formed the derivative in the presence of o-phenylenediamine. For this reason an aliquot of each extract was treated with oxalate decarboxylase prior to derivitization in order to determine the amount of derivatizable compound(s) other than oxalic acid contained in each. To determine the oxalic acid content of a sample, the calculated concentration of the enzyme treated aliquot (sample conc. ppm) was subtracted from the calculated concentration of the untreated aliquot.

It was established that the sample material had no adverse effect upon the enzyme by spiking samples of each new plant type with a known amount of oxalic acid. In effect, the spiked enzyme treated sample could have no more derivative than the unspiked enzyme treated sample unless an enzyme inhibitor was in the sample. None were found in alfalfa and fescue.

Fractionation of derivitized material was so complete that virtually all interfering compounds were removed before injection into the HPLC. This not only resulted in very uncomplicated elution profiles but also very short analysis times (5 minutes) and no need for solvent changes in order to remove late eluting peaks. The fact that a relatively large derivative fraction was collected from the disposable columns (1.5 X the amount loaded) contributed to excellent recovery of the derivative (100.15%; S.D. = 2.24; N = 10).

A sample's apparent concentration of oxalic acid in ppm at the 40 ml extract stage was calculated from its coinciding regression equation and multiplied by the dilution factor to arrive at the calculated value of oxalic acid in air dried plant matter.

Samples of ground alfalfa and tall fescue were analyzed in triplicate. All initial calibration and recovery determinations were made with sodium oxalate which is used in most reports in the literature. Our initial analysis of unknown forage samples with water extraction resulted in oxalic acid values for tall fescue equal to or greater than values for alfalfa. Such values were not expected and were not consistent with values in the literature. Subsequently, it was decided to use enzymatic incubation of sample extracts in order to gain a more thorough measurement of oxalic acid in the extract.

At another point of development in this technique, we questioned whether all forms of oxalate were being adequately extracted from the forage samples. Table 1 illustrates the comparison of acid and water extraction. Two to four times more oxalic acid was extracted with acid than water. The standard deviation ranged from .002 to .035.

Table 2 and 3 illustrate the recovery of sodium or calcium oxalate with either water or acid extraction. Recovery of calcium oxalate with water extraction was almost nil, confirming that either it is insoluable in water or it attaches tightly to forage particles. Sodium oxalate was nearly 100% extractable with water and about 85 to 90% extractable with 1 N HCl acid.

Calcium oxalate extraction in acid (table 2 and 3) was greatly improved compared to water extraction. Recovery of calcium oxalate extracted with acid appears to be about 80%. The low values of 35 & 50% in table 2 cannot be fully explained. Total oxalic acid can be extracted and analyzed in small samples (250 mg) of forage but must be extracted with acid. Water may also be used to extract oxalic acid but values are usually lower than when acid is used and the biological implications of water extracted oxalic acid is currently unknown.

Table 1. Oxalate content of alfalfa and tall fescue extracted with either acid or water (percent of air dry matter).

	Extract			
Forage	Water (%)	SD*	Acid (%)	SD*
Alfalfa	0.12	.035	0.830	.019
Fescue	0.18	.002	0.209	.031

* Standard deviation of three determinations.

Table 2. Recovery of sodium and calcium oxalate from spiked samples of alfalfa and tall fescue (percent recovery of added oxalate).

	Water Extract		Acid Extract	
Forage	Sodium Oxalate	Calcium Oxalate	Sodium Oxalate*	Calcium Oxalate*
Alfalfa 1	107.7	-3.6	81.7	50.8
Alfalfa 2	101.4	3.9	84.0	35.5
Fescue 1	98.2	3.4	85.5	71.4
Fescue 2	91.9	-5.6	91.1	72.8

* Sodium oxalate added just prior to derivitization and calcium oxalate added to extraction flask with sample.

Table 3. Recovery of sodium and calcium oxalate from spiked samples of alfalfa and tall fescue extracted with acid (percent recovery of added oxalate).*

Forage	Sodium Oxalate	Calcium Oxalate
Fescue Filtered	88.6	68.1
Fescue Centrifuged	109.5	99.9
Alfalfa Filtered	118.8	95.8
Alfalfa Centrifuged	73.7	88.4

* Each value is mean of three determinations.

NEW PYCNOMETRIC APPROACH TO MEASURE SPECIFIC GRAVITY OF FORAGE SAMPLE

M. WATTIAUX, D.R. MERTENS AND L.D. SATTER

INTRODUCTION

Specific gravity (SG) is recognized as a factor influencing stratification of rumen contents and the rate at which partially digested forage particles escape from the reticulorumen. Functional specific gravity (FSG), based on the sample dry matter and the internal air or gas pockets, does not account for the effect of sample moisture on SG. As currently defined, a change in FSG cannot be partitioned between dry matter (DM) specific gravity and sample gas content. Unfortunately, techniques currently available do not permit determination of a SG of physiological significance, that is SG of a sample undergoing initial solubilization and hydration by rumen fluid followed by microbial fermentation.

The objectives of this experiment were to test the effect of sample water content on FSG and to validate a new approach for describing more thoroughly the specific gravities of a sample.

MATERIALS AND METHODS

A sample's SG can be calculated from the weight and volume

(measured as the weight of the same volume of distilled water at a chosen reference temperature) of either the dry matter only (dry specific gravity, DSG), the dry matter and water (compact specific gravity, CSG) or the dry matter, water, and gas content (unit specific gravity, USG) (Fig. 1).

Two particle sizes (PS) were obtained by grinding alfalfa hay through a 2 or 8 mm Wiley mill screen and collecting the dry sieved material ranging from 0.3 to 0.6 mm and 0.85 to 1.18 mm, respectively. For each PS, material was used "as-is" (high dry matter, HDM) or a known amount of degassed distilled water was added to lower the dry matter content (LDM). Fifty ml boiling flasks, modified to enable a precise volume measurement, were used as pycnometers. The displacement solution was degassed clarified rumen fluid (DCRF) at room temperature.

For each PS-DM combination, CSG was determined on a sample which was vacuumed at 720 mm Hg for 10 min to remove all gases. FSG and USG were determined on a second sample not subjected to vacuuming. DSG and the fractional volumes of solid, liquid, and gas were then calculated.

RESULTS AND DISCUSSION

The SG of DCRF was 1.009. Sample DM, DSG, CSG, USG, and FSG for the 0.85-1.18 mm PS only are presented in table 1. Functional SG is insensitive to changes in sample moisture content (0.74 vs. 0.70 for HDM and LDM, respectively).

Unlike FSG, USG accounts for sample moisture content. Water in the HDM and LDM was 4.3 and 72.0% of the total volume, respectively (Fig. 2). Therefore, USG is slightly higher than FSG at HDM (0.75 vs. 0.74) and the difference is larger for LDM (0.92 vs. 0.70). Furthermore, because the SG of added water is 1.00, an increase in water content brings USG closer to 1.00 (0.75 vs 0.92).

Dry SG, which only accounts for the SG of the DM, is unaltered by water in the sample (1.55 vs. 1.53). However CSG, which represents the SG of DM and water is close to DSG when water makes up a small fraction of the sample volume (1.50 vs. 1.55 at HDM) and decreases to near 1.00 when water is a large fraction of the sample volume (1.08 at LDM).

CONCLUSION

Functional SG is only indirectly affected by the sample water content. However, because a SG of physiological significance must account for any differences in gas, liquid or dry matter volumes, USG is proposed as a better estimate of "functional specific gravity" in vivo. Moreover, CSG and DSG will be necessary to interpret correctly any change in USG that a forage sample might undergo during the process of ruminal digestion.

TREATMENT	DRY MATTER (%)	DSG	CSG	USG	FSG
HDM	94.3	1.55 0.06 (1)	1.50 0.05	0.75 0.10	0.74 0.10
LDM	20.8	1.53 0.07	1.08 0.01	0.92 0.07	0.70 0.15

Table 1. Effect of water content on SG of alfalfa hay(0.85 – 1.18 mm.).

(1) Standard deviation of the means, n=4

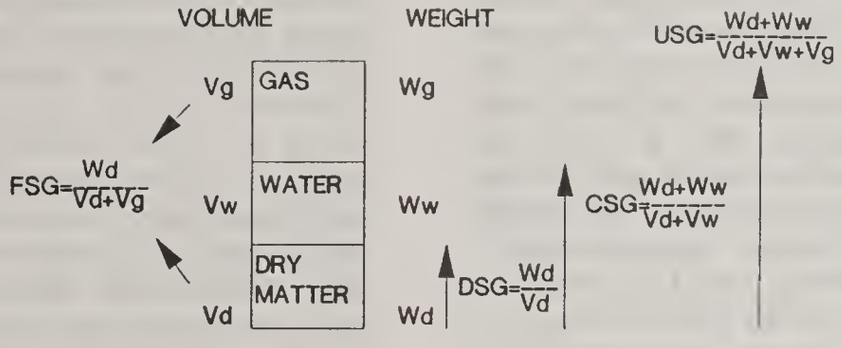


Figure 1. Specific gravity (SG) terminology: Vg, Vw, Vd are the volumes (measured as the weight of the same volume of water at reference temperature), and Wg, Ww, Wd are the weights of gas, water, and dry matter respectively. The gas fraction is assumed weightless (Wg=0) and water SG=1 (Ww=Vw). FSG: Functional SG, DSG: Dry matter SG, CSG: Compact SG, USG: Unit SG.

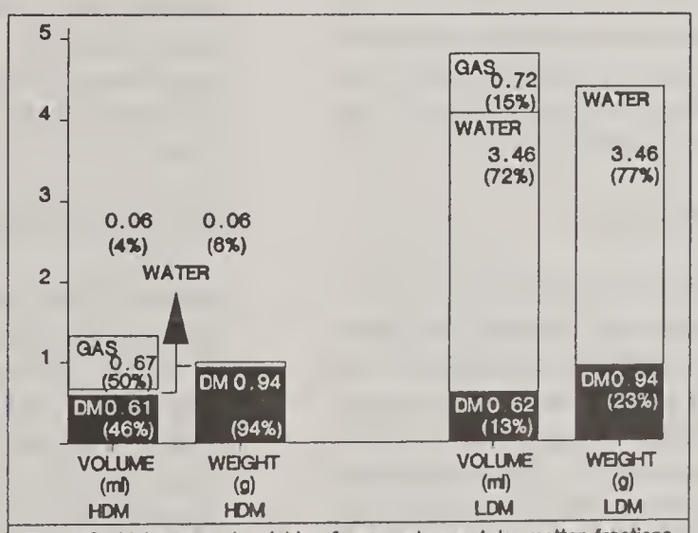


Figure 2. Volumes and weights of gas, water, and dry matter fractions of alfalfa hay with or without water added (LDM, HDM respectively)

COMPARISON OF ESTIMATES OF RUMINAL PROTEIN DEGRADATION BY IN VITRO AND IN SITU METHODS

G.A. BRODERICK, R.J. WALLACE, E.R. ØRSKOV AND L. HANSEN

INTRODUCTION

Dietary proteins vary greatly in ruminal degradability and new feeding systems place emphasis on quantifying ruminal protein degradation and escape (e.g., Ruminant Nitrogen Usage, NRC, 1985). Therefore, it is now necessary to rapidly and accurately assess ruminal degradability of feed proteins. The procedure gaining widest application is the in situ bag technique where the protein is suspended in fiber bags within the rumen, and N loss is used to determine rate and extent of protein degradation. Although used extensively, in situ techniques have been criticized. A major problem with most in vitro systems has been incomplete recovery of degradation products. We recently reported on a revised in vitro method which includes inhibitors of microbial N metabolism to allow quantitative recovery of protein degradation products (Broderick, Brit. J. Nutr. 58:463, 1987). The purpose of this research was to compare the inhibitor in vitro (IIV) method with a conventional in situ procedure for estimating rates and extents of ruminal protein degradation.

MATERIALS AND METHODS

The following proteins were studied: casein was used as purchased (from Sigma Chemical Co.) or after precipitation and freeze-drying; pot ale syrup and spent wash were by-products from Scotch whisky production; four samples of fish solubles (fish solubles I to IV) and one sample each of "non-ruminant" and "ruminant" fish meal; and soybean meal, sunflower meal, linseed meal, rapeseed meal, copra meal, and meat and bone meal (blood meal and feather meal were found to be degraded too slowly to give reliable degradation rates). All samples were analyzed for total Kjeldahl N and dry matter; total amino acids (TAA) were determined in acid hydrolysates. The proteins were also assayed for water soluble N and for low molecular weight N compounds using dialysis tubing with a 12,000 molecular weight cut-off. The in situ protein degradation procedure was that outlined by Ganev et al. (J. Agr. Sci. (Camb.) 93:651, 1979). Five g air-dry samples were weighed into 10X17 cm nylon fiber bags with pore size of of 50X80 microns. Bags were suspended for periods of 4, 8, 16, and 24 h in the rumens of sheep fed ryegrass hay. Rates of ruminal protein degradation were estimated by the IIV method (Broderick, 1987). In this procedure, the inhibitors hydrazine and chloramphenicol are incorporated into the medium to give quantitative recovery of ammonia and TAA produced during protein degradation.

RESULTS AND DISCUSSION

Analytical and in vitro degradation data obtained for all 16 proteins are in Table 1. Degradation rates for the eight soluble proteins obtained with the IIV method were very rapid, typically about .5/h or faster. Exceptions were the proteins in pot ale syrup and spent wash (Table 1), which were degraded

at .103 and .145/h, respectively. Both are by-products from Scotch whisky distilleries, and these slower rates may reflect largely the degradation of soluble barley and yeast proteins. Degradation was not solely a function of protein solubility (Table 1). Degradation rates for soluble proteins ranged from values similar to oilseed meals (about .1 to .15/h) to rates as high as .8/h, reflecting the lack of homogeneity of degradability among soluble proteins. Very slow degradation rates have been observed by others for certain soluble proteins. Slow rates for soluble proteins have been attributed to intramolecular cross-linking within these proteins.

A comparison of IIV and in situ degradation data obtained with the seven protein meals (which are largely insoluble) is in Table 2. Repeatable data were obtained with both methods; degradation rates ranged from .042 to .244/h and .014 to .067/h by IIV and in situ procedures, respectively. Although rates obtained using the in situ method were much slower than by IIV (average 36%), the same ranking of these proteins was obtained using either method.

Rates determined by in situ methods may be expected to be lower than actual in vivo rates for at least three reasons: 1) microbial contamination within in situ bags tends to reduce apparent rate of N loss; 2) the microbial population inside the bag is restricted to numbers fewer than that of the surrounding digesta; 3) rapid efflux from the bags of soluble, degradable protein results in residual protein which is more slowly degraded than the protein as a whole. These difficulties do not occur with the IIV system. Its degradation rate is derived from appearance of ammonia plus amino acids (from both soluble and insoluble proteins) as a consequence of degradation.

Estimated extents of degradation (Table 2), computed from in situ and IIV data using ruminal passage rates of .02, .05 and .08/h, were more similar than degradation rates, and were highly correlated to each other (r^2=.830, n=21). Overall, mean in situ degradabilities averaged 83% of those estimated from IIV data.

Improved correspondence of results is due to the greater influence of the rapidly degraded fraction (the proportion of total N rapidly leaving in situ bags) in the computation of in situ degradations. Fraction 'a' (a = 1 - b, where b is the intercept value) averaged 23.7% of total N, while the corresponding fraction 'a' from the IIV assay (the proportion of total protein "degraded" at 0-h), averaged only 11.0%.

Estimates of ruminal degradation (based on in vivo data) published in Ruminant Nitrogen Usage (NRC, 1985) for fish meal (well-preserved), soybean meal, linseed meal, sunflower meal, rapeseed meal and meat and bone meal were 20, 72, 56, 76, 77 and 40%. Degradability was not given for copra meal. Fish meal which is "stale at processing" was reported to have a degradability of 52%, similar to the mean of IIV and in situ estimates of 54% from this study. It is difficult to assess technique reliability from so few data; however, the overall similarity of our results to published protein degradabilities indicates both the IIV and in situ methods were satisfactory.

TABLE 1. CHEMICAL ANALYSES AND INHIBITOR IN VITRO (IIV) DEGRADATION DATA FROM 18 PROTEIN SOURCES

Protein	Total-N (DM Basis), %	TAA,[a] umol/mgN	WSN,[b] %	Low MWN,[c] %	IIV Oegradation rate (k_d),h^{-1} (±SE)	IIV intercept (B), %	IIV degradability,[d] %
Casein (Sigma)	15.53	46.5	100	ND	.489 ±.054	101.7	91
Casein (freeze-dried)	13.43	48.2	100	8.0	.813 ±.110	98.4	94
Pot ale syrup	4.55	35.8	100	40.8	.103 ±.010	81.9	73
Spent wash	4.89	35.3	100	21.5	.145 ±.010	81.8	79
Fish solubles I	11.86	50.7	100	28.7	.594 ±.050	92.7	93
II	12.26	49.0	100	22.1	.550 ±.046	92.5	92
III	11.34	49.3	100	24.6	.584 ±.052	95.7	92
IV	11.19	50.0	100	24.9	.538 ±.048	93.1	92
Fish meal (nonruminant)	11.05	50.7	46.5	4.7	.078 ±.009	78.5	69
Fish meal (ruminant)	11.04	48.9	27.0	3.3	.042 ±.009	81.9	55
Soybean meal	7.62	43.8	43.7	10.0	.166 ±.019	92.7	79
Linseed meal	5.69	47.8	29.0	1.8	.244 ±.034	94.7	84
Sunflower meal	4.53	44.6	30.7	9.0	.058 ±.008	87.7	59
Rapeseed meal	6.40	42.0	29.7	10.0	.124 ±.011	85.9	75
Copra meal	3.25	42.1	39.3	12.7	.050 ±.007	92.2	54
Meat & bone meal	7.67	52.6	29.3	8.0	.056 ±.011	88.2	58

[a] TAA=Total amino acids; SE = standard error of the mean; ND = not determined.

[b] Water soluble N, proportion of total N. Values of "100" were based on dry matter solubilities and were not determined directly.

[c] Low molecular weight N; proportion of total N in compounds of molecular weight less than 12,000 daltons.

[d] Extent of degradation of protein, % = (100-B) + $B[k_d/(k_d + k_p)]$, where $B \leq 100$ and the assumed ruminal passage rate, k_p=.05/h (Broderick, Brit. J. Nutr. 58:463, 1987).

TABLE 2. PROTEIN OEGRAOATION PARAMETERS OBTAINEO WITH INHIBITOR IN VITRO (IIV) AND IN SITU INCUBATIONS

Protein	Degradation rate[a]		Intercept[b]		IIV degradability at ruminal passage of (h^{-1})[c]			In situ degradability at ruminal passage of (h^{-1})[d]		
	IIV (k_d)	In situ (c)	IIV (B)	In situ (b)	.02	.05	.08	.02	.05	.08
	------h^{-1}-----		----------%----------							
Fish meal (ruminant)	.042	.014	81.9	68.6	74	55	46	60	46	42
Soybean meal	.166	.067	92.7	85.6	90	79	70	80	63	53
Linseed meal	.244	.077	94.7	79.8	93	84	77	84	69	59
Sunflower meal	.058	.032	87.7	81.0	78	59	49	69	51	42
Rapeseed meal	.124	.036	85.9	82.3	88	75	66	71	52	43
Copra meal	.050	.017	92.2	76.0	74	54	43	59	43	37
Meat & bone meal	.056	.017	88.2	60.6	77	58	48	67	55	50

[a] Fractional protein degradation rates determined by IIV (k_d) and in situ (c) methodology.

[b] Antilog of intercept from regression on time of log of fraction remaining undegraded.

[c] Extent of protein degradation estimated from IIV data (Broderick, 1987) at three assumed ruminal passage rates (k_p) using the equation: protein degradability, % = (100-B) + $B[k_d/(k_d + k_p)]$.

[d] Extent of protein degradation estimated from in situ data (Ørskov and McOonald, J. Agr. Sci. (Camb.) 92:499, 1979) at three assumed ruminal passage rates (k) using the equation: protein degradability, % = (100-b) + $b[c/(c+k)]$.

U.S. DAIRY FORAGE RESEARCH CENTER
ANNUAL DAIRY OPERATIONS REPORT, FEBRUARY 1988

L.L. STROZINSKI

The research center herd has continued to grow in the past year and is nearing its full capacity with a present total herd count of 530 head (260 cows and 270 herd replacements). The DHI rolling herd average peaked at 17,187 pounds of milk and 629 pounds of fat during the year but has slipped downward to the present average of 16,330 pounds of milk and 593 pounds of fat. I feel that this is a reflection of research pressure, a shortage of alfalfa haylage last spring, a very hot summer and a tendency toward a larger and younger herd. Current daily production average per cow on 226 milking animals is 61.5 pounds. Calving projections indicate that the milking herd will move to 250 by the end of March. I expect the DHI average to turn to an upward trend in the near future. Milk and animal sales income for fiscal year 1987 totaled $454,910 and $63,851, respectively. With some uncertainty of future milk and animal prices I have projected fiscal year 1988 milk and animal sales income to be $475,000 and $63,000, respectively.

I feel that the overall quality of the herd has continued to increase. This month the Holstein Association cow index report listed 84 of our milking age animals as quality performers with cow type-production indices in the top 30% of all Holsteins summarized. Three animals were listed on the locator list which includes the top 5,000 of all cows summarized.

The reproductive status of our herd continues to be good. At present, average days open for the herd is 103 days. Average age at freshening for first calf heifers is 24 months. Therefore, we continue to have a large number of quality herd replacements. Calf mortality continues to be very low with a loss of only 4 out of 171 female calves born during the last year. This year we marketed three dairy heifers in consignment sales conducted by the Badger Dairy Club of Madison and the Pioneer Dairy Club of Platteville. Marketing and culling strategies for surplus dairy stock in the future are being developed.

Conversion of the computerized dairy record system to the Dairy Comp 305 system was completed during the past year. Additions and enhancements of the system have also been made. A new daily milk record system has recently been implemented which allows retrieval of specific daily milk production data in a format which directly loads to spreadsheets. The overall system has functioned extremely well and is a great improvement over the previous system.

During the summer of 1987 we had the opportunity to participate in a large APHIS/ARS cooperative blue tongue research project. The purpose of the study was to determine if the blue tongue virus can be transmitted via embryo transfer. One hundred and seventy beef cattle carrying embryos from blue tongue infected donors were pastured at the Dairy Forage Center to avoid contact with the Culicoides varipennis gnat which is known to carry the blue tongue virus in the south.

Recently we entered into another cooperative project with the State Laboratory of Hygiene for the Center of Disease Control and U.S. Public Health Service. This project involves dosing of three dairy animals with lead three times a year and bleeding the animals for several days after dosing. Blood is then sent to numerous laboratories throughout the U.S. for a proficiency testing program which will improve the ability of laboratories to accurately detect lead poisoning in children at risk.

Currently there are five research feeding trials in progress involving 180 dairy animals and 24 sheep. A total of 35 different diets are being prepared and fed daily. During the past year no less than eleven major feeding trials have been conducted.

Handling of manure continues to be a problem at the Center. A great deal of effort was put into our manure separation system by Dr. Koegel and his staff over the past year. Another separator was purchased and remodeled in an attempt to improve its overall performance and decrease operating costs. After approximately nine months of not separating manure, we started using the remodeled unit in October. Total output of the new unit is higher than the old unit due to some design changes which have resulted in considerably less machine plugging. The complexity of the unit and the large number of moving parts have unfortunately kept downtime and maintenance costs higher than we would like to see. Continued efforts are being made to improve the separation process. Manure solids are again being used to bed the majority of our animals. Dr. Muck plans to investigate methods of improving the composting process of the manure solids in the near future.

As one would expect, the increase in livestock numbers and research load has put an increased demand on the labor force. The labor force in the dairy operation is structured to include 15 experimental farm laborers, 2 herd assistants, one herd supervisor and one maintenance mechanic. At the present time there are 3 vacancies in the crew which are being covered by limited term employees. One vacancy is temporary due to an educational leave of absence. The other two are due to a resignation and a newly created position. Recruitment for these two vacancies is in progress. Since the herd size will be plateauing soon I hope that additional full-time positions will not be needed in the future. The field crew has been and continues to provide a great deal of assistance to the dairy operation during the winter months.

In summary let me say that the past year has been a challenging but productive year for the dairy operation. Credit for the output of quality support to the research staff must be given to the labor force. I believe that as a public research institution the Dairy Forage Center has one of the highest outputs of quality dairy research support in the nation. The challenge for the future will be to continue this support while staying within our fiscal restraints.

U.S. DAIRY FORAGE RESEARCH CENTER
ANNUAL FIELD OPERATIONS REPORT, FEBRUARY 1988

B.C. VENUTO

The summer of 1987 was a frustrating season for harvesting quality forage to meet the research and production requirements of the Center. Winterkill coupled with an unusually early and dry spring aggravated by heavy insect pressure, greatly affected our alfalfa. Quality was good but yields were disappointing. We were, however, successful at achieving most of our goals even though some had to be modified along the way.

In January of 1984 we had 301 animals (50% milking and dry) and in January 1988 we had 525 animals (49% milking and dry). Our forage acreage during the same period increased from 385 acres to 618 acres. This shift in acreage as well as an increase in research demand since 1984 has resulted in more work load on the field crew. They have responded quite well to the challenge of not only harvesting 500+ acres of quality forage but also meeting the specific feed needs of the research staff. In 1987 the permanent field crew staff had accumulated an average of 200 hours of compensatory time by November 1st. This time was not the result of working holidays as much as it was working long days, often going until 9:00 or 10:00 at night. With their help and cooperation we were able to make 1987 a good year and hope to make 1988 even better.

CHALLENGES FOR 1988

One objective that we have been striving for is better utilization of our manure resource. In 1987 we hauled and spread 3.4 million gallons of liquid manure with a fertilizer value of $30,000 to $34,000. By using surplus army trucks we have been able to keep our cost for hauling less than half the local custom rate. In addition we are applying manure on fields that need it and are starting to see the benefits in our soil test and our reduced commerical fertilizer usage.

In 1988 we will have a second surplus army truck equipped to haul manure. This will result in improved labor and fuel efficiencies. In addition, we will continue to search the Federal surplus for quality used equipment that will either replace aging equipment or provide improved resources during peak demand periods.

During the last four years we have continually upgraded our field record keeping system. In 1988 we will be implementing a new computerized system developed through the cooperative effort of the U.S. Dairy Forage Research Center and the University of Wisconsin Agricultural Research Stations. We hope to see this program implemented by most of the Agricultural Research Stations in Wisconsin as well as the Dairy Forage Center.

Good management requires good records. It is imperative that we keep track of the what, when, where and how much so as to analyze and improve our efficiency.

Those of us who work at the Dairy Forage Center Field Facility take great pride in accepting the

challenge of attaining and maintaining a level of performance second to none. The future demands on us to support our excellent research staff coupled with the fiscal reality of dwindling resources will be our greatest challenge yet. By maintaining a spirit of cooperation and pride among our researchers, our managers and our workers, I believe we can be successful.

U.S. DAIRY FORAGE RESEARCH CENTER
LAND USE COMPARISON

CROP	1984 ACRES	1988 ACRES
Corn	493.7	232.0
Wheat	46.0	60.0
Forage		
Grass		
Ryegrass	2.0	3.0
Fescue	-	3.0
Canarygrass	-	3.0
Timothy	-	3.0
Orchardgrass	-	3.0
Bromegrass	-	3.0
Big Bluestem	-	3.0
Grass Total	2.0	21.0
Legumes		
Alfalfa-New Seeding	57.0	143.5
Alfalfa-Established	280.0	430.1
Trefoil	-	8.0
Red Clover	10.0	8.0
Ladino	-	3.0
Crown Vetch	-	3.0
Legume Total	347.0	597.6
Legume/Grass		
Ryegrass/Alfalfa	36.0	-
Small Plot Area	5.0	18.0
TOTAL CROP ACRES	929.7	926.6
Fallow	99.0	-
Pasture	271.0	271.0
TOTAL ACRES	1299.7	*1197.6

* 85 acres returned to Badger after 1986 season.

U.S. DAIRY FORAGE RESEARCH CENTER
1987 CROPS & YIELDS

CROP	ACRES	YIELD/ACRE
Corn		
Grain	90.6	146.7 Bushels
H.M. Ear	155.3	4.7 Tons (wet)
Silage	80.3	13.92 Tons (wet)
Winter Wheat	60.0	77.0 Bushels
Alfalfa		
New Seeding		
Spring	153.3	1.33-2.77 Ton (D.M.)
Fall	43.5	-
Established	252.2	3.2 Ton (D.M.)
Harvested, plowed and planted to wheat	60.0	2.12 Ton (D.M.)

CORN

Corn yields were also slightly less than last year with short season corn suffering the most. The rains came too late to insure complete pollination for the early corn whereas the long season corns benefited both by the increased growing degree days and the mid summer rains.

WINTER WHEAT

Wheat yields were much better than anticipated. Winter wheat was seeded quite late in 1986 due to wet fall conditions but survived the winter very well. Our rotation for winter wheat is to harvest an older stand of alfalfa three times, manuring after each cutting, plow in August and seed winter wheat in early October. No additional fertilizer is applied.

ALFALFA

1987 was an extremely poor year for forage. Fields that were harvested in the Fall of 1986 suffered extensive winter damage. Second crop yields were 93% of third crop yields due to an extremely hot dry spring. Timely rains salvaged third crop and saved new seedings. Second harvest yields of new seeding were equal to or up to 50% greater than the first cutting of new seeding. Alfalfa weevils and leafhoppers were the worst they have been for many years. Growing degree days as of June 28 were 272 days (modified base 50) ahead of normal while rainfall was several inches below normal.

A fall seeding of alfalfa was made after harvesting winter wheat. The field was manured heavily, then offset disked and pulvimulched prior to seeding. Poast was applied in September to control volunteer wheat. The stand looked very good last fall.

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